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CONTENTS

The Intestinal Villi and Their Circulation in Relation to Absorption and Secretion of Fluid. *Herbert S. Wells and Ralph G. Johnson* 387

Changes of the Carbohydrate Metabolism of the Heart Following Coronary Occlusion. *H. E. Himwich, W. Goldfarb and L. H. Nahum* 403

The Significance of Frequency, Number of Impulses and Fiber Size in Vasomotor Responses to Vagus and Depressor Nerve Stimulation in the Rabbit. *George H. Bishop, Peter Heinbecker and James O'Leary* 409

The Effect of Insulin in Conjunction with Posture on the Blood Concentration in Depancreatized Dogs. *Alice M. Waterhouse and Elaine P. Ralli. With the technical assistance of Onna Reinhardt* 422

Nutritional Significance of Vitamin A Throughout the Life Cycle. *Esther L. Batchelder* .. 430

"The Fat Metabolism Hormone" and Hyperglycemia. *Benjamin Harrow, I. M. Chamelin and Abraham Mazur* 436

Studies of the Nervous System in Deficiency Diseases: Experimental Black Tongue. *H. M. Zimmerman, G. R. Cowgill, W. W. Bunnell and Margaret Dann* 440

What Constitutes a Lethal Reduction of Temperature? *F. K. Jackson and A. Alonge* 447

Studies on the Action of the Alkali Iodides. *Jacob John Westra* 450

The Calcium Content of Frog Nerve. *S. R. Tipton* 457

The Permeability of the Blood Capillaries to Lipoids. *Alexander Marble, Madeleine E. Field, Cecil K. Drinker and Rachel M. Smith* 467

Hypoglycaemia Hypophysiopriva. *William Mahoney* 475

Spiral Propulsion of a Bolus in the Intestine. *P. E. Reid, A. C. Ivy and J. P. Quigley* 483

A Study of the Blood Volume of Adrenalectomized Dogs. *W. W. Swingle, H. M. Vars and W. M. Parkins* 488

Tissue Metabolism of Warm-Blooded Animals at Normal and at Febrile Temperatures. *M. Elizabeth Marsh* 502

The Responses to Changes in Environmental Temperature after Removal of Portions of the Forebrain. *James O. Pinkston, Philip Bard and David McK. Rioch* 515

The Clearance of Creatine in Dog and Man. *Robert E. Pitts* 532

The Clearance of Creatine in the Phlorizinized Dog. *Robert F. Pitts* 542

The Influence of Neutral Sodium-Salt Solutions on Chemical Stimulation. *Iping Chao* .. 550

The Cold Stimulation and the Influence of Neutral Sodium-Salt Solutions on the Cold Stimulation. *Iping Chao* 561

The Viscosity of the Blood of Pigeons in the Quiet State and Following Excitement. *L. B. Nice and David Fishman* 569

The Composition of Edema Fluid and Lymph in Edema and Elephantiasis Resulting from Lymphatic Obstruction. *Cecil K. Drinker, Madeleine E. Field, J. William Heim and Octa C. Leigh, Jr.* 572

The Effect of Histamine on Jejunal Secretion. *A. L. Berndt and I. S. Ravdin* 587

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THE INTESTINAL VILLI AND THEIR CIRCULATION IN RELATION TO ABSORPTION AND SECRETION OF FLUID[1]

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From the Department of Physiology, Vanderbilt University School of Medicine, Nashville

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In preceding papers (1, 2) data were presented which indicate that the absorbing force of the intestine, with respect to fluid, is provided by the osmotic pressure of proteins in the tissue fluids of the villi. This absorbing force may be counteracted or even overbalanced by the application of a sufficient negative hydrostatic pressure to the intestinal contents. The withdrawal of fluid from the tissues, which may be brought about in this manner, has been described as representing a type of ultrafiltration. In theory, ultrafiltration should occur, also, on the application of a sufficient positive hydrostatic pressure to the tissue fluids, but the measurement of this tissue pressure must await the development of more refined methods than are at present available. Visual examination of the mucosa during absorption and secretion of fluid indicates, however, that the tissue pressure is increased during secretion and it appears very likely that ultrafiltration of fluid from the tensely swollen villi is an important factor in the process. Detailed observations of the form, size, and movements of the villi, and of the reactions of the local blood vessels have been made, especially the reactions of hyperemia which are so easily provoked by a variety of mildly irritating agents. The observations point very definitely to a consistent and logical interpretation of the phenomena of intestinal secretion, as they occur in the normal animal. The findings lead us further to conclude that the villus-pump theory of intestinal absorption, which is still held by some investigators, is based on faulty observations. Consequently our observations support the assumption that the physical factors of osmotic and hydrostatic pressures, under the regulation of the local vasomotor-villus reactions, are sufficient to account for all of the facts which are definitely known with regard to the transfer of fluid through the gut wall, in either direction.

[1] Aided by a Fluid Research Fund of the Rockefeller Foundation.

METHODS. Dogs, anesthetized by the intravenous injection of 0.25 to 0.5 gram of sodium barbital per kilo, were used as experimental animals. The gut was examined during various phases of digestion and after fasting for periods up to 24 hours. In most of the experiments a loop of the upper jejunum was employed. Such a loop, having been selected to provide a convenient distance between branches of the mesenteric vessels, is pulled carefully through the slit in the lower plate, *A*, of the special intestinal

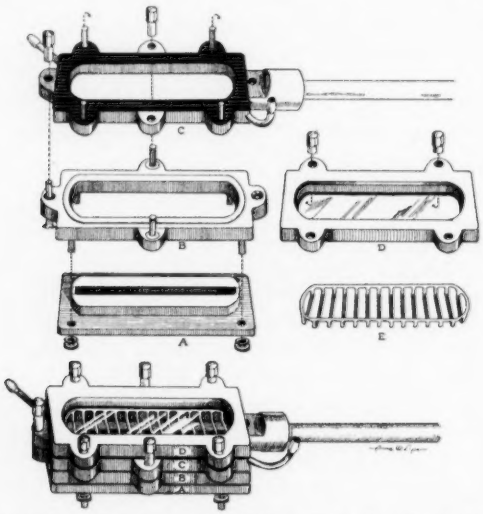

Fig. 1. *Intestinal clamp for observation of the villi during absorption or secretion of fluid.* The assembled clamp is shown at the bottom. Plate *A*, which contains a slit for the passage of the mesentery, is telescoped into the lower portion *B*, of the clamp. Screw posts and nuts at the corners of *B* allow the adjustment of the supporting surface of *A* in relation to the thickness of the gut wall. The upper surface of *B* and the lower surface of *C* are provided with rounded ridges, 3 mm. wide and 1 mm. high, which act as the jaws of the clamp. Plate *C* provides the sides of the absorption chamber, which is 2.5 cm. wide, 10 cm. long and 1 cm. in depth. The cover, *D*, which consists of a glass plate held in the aluminum frame by means of an asphalt cement, closes the chamber with a water-tight joint that is provided for by the rubber gasket shown at *C*. The tray, *E*, lies in the chamber with its cross-wires on the mucosa and its upper edges lying against the glass plate of the cover.

clamp which is illustrated in figure 1. Although this clamp was mentioned in a previous communication (1), the details of its construction and use may warrant further description. The loop of gut is then doubly ligated at either end with cloth tapes and cut through between the ligatures. The loose ends of gut are pulled back through the slit and allowed to project just outside the abdominal wound. The isolated loop is then stretched

lengthwise of the clamp so that the ligated ends project beyond the ends of the clamp. The gut is slit open lengthwise along a line which is directly opposite the attachment of the mesentery. Bleeding is controlled and the gut is prevented from curling up by attaching large spring paper clips (obtainable at any stationery store) to the edges. The upper plate, *C*, of the clamp is then laid over the gut and fastened in place, pressure being applied by means of the two lateral thumb nuts. A screw is then inserted at each end—holes being punched through the whole thickness of the gut by the screws themselves—and thumbscrews applied. These four screws must be tightened several times during the next few minutes, in order to take up the space which is vacated by the muscular layer of the gut as it gradually becomes thinner at the line of occlusion between the rounded ridges of the upper and lower plates (*B* and *C*). There is no hemorrhage after the clamp is once applied, and when it is tightened up in successive steps, as described, the chamber becomes water tight. The mucosa is then washed and wiped clear of blood and mucus, using warm physiological salt solution for the rinsing. The supporting grid or tray, *E*, is then put in, and the rubber gasket and glass cover, *D*, applied. The cover is screwed tightly in place by means of four nuts. The chamber is connected by its two outlet tubes to a waste bottle and to the 10 cc. burette which is used to adjust the intra-intestinal pressure and to measure the volume changes. Warm salt solution is run in and the clamp tilted so as to allow all the air bubbles to run out through the rubber tube which leads to the waste bottle. Both inlet and outlet tubes are then clamped off and the intestinal clamp is fixed in a horizontal position just above the abdominal wall. For this purpose the supporting rod is screwed into its housing in the upper plate of the clamp. A heavy support stand serves to hold the apparatus in the proper position. By means of a pair of levelling bulbs the atmospheric pressure level—the level of the upper surface of the mucosa of the isolated loop—is determined with reference to the scale reading of a meter stick which stands vertically beside the burette. Unless otherwise stated, the intra-intestinal pressures employed in the series of experiments varied from atmospheric pressure to minus 4 cm. of saline, but a constant pressure was maintained during any given observation.

Absorption (decrease in volume) and secretion (increase in volume) have been recorded by means of a piston recorder connected to the upper end of the burette. It was of course necessary to allow for changes in volume due to changes in the tone of the gut in order to interpret the tracings correctly. Blood pressure from the carotid artery and time intervals of 1 minute were also recorded on the kymograph tracing. The graph was marked frequently by means of a signal magnet so that the record could be compared at numerous points with the descriptions of the appearances of the villi, and other data, recorded in the protocols.

When the animal is in good condition and the gut is carefully handled it has been found that absorption and villus movements may continue, with little evidence of change, for as long as 6 to 8 hours. By keeping the room temperature at about 30°C. it is found that the animal maintains its normal temperature and the temperature of the fluid in the clamp does not fall below 36°C.

The movements of the villi have been observed with a binocular microscope at various magnifications from 10× to 100×, using an arc light for illumination of the field. Most of the observations were carried out by reflected light, but in each experiment it was possible to study a number of villi which would be lying close to the cross-wires of the grid, and as these wires were highly polished they would reflect a strong light through the overlying villi; a condition which greatly facilitated the observation of the blood flow, for the vessels would stand out very clearly against the bright background of the nearly transparent tissues of the villi.

For the observations on isolated villi, small pieces of mucosa were sliced off from various parts of the gut with a razor blade and the villi were teased out in saline on a microscopic slide by the aid of a probe or needle. In order to keep the preparation warm during the observations the microscope was housed in a thermostatically controlled box at body temperature.

DESCRIPTION OF THE FINDINGS. *Observations indicating the incorrectness of the villus pump theory of intestinal absorption.* All observers agree that there are three types of villus movements: the tonic retraction, usually involving more than one villus at a time, is ordinarily associated with contraction of the muscularis mucosae, and may be brought about either by sympathetic stimulation or by various types of local irritation of the mucosa; the whipping, swaying movements of individual villi, without shortening; and the rhythmic retractions in which the villus may shorten as much as half its total length. In the third type any individual villus retracts suddenly and then relaxes either suddenly or gradually. It is this type of movement which has been considered to represent a pumping action, and this mechanical activity has been invoked as a more or less essential feature of the mechanism for intestinal absorption of fluid (4, 5, 6, 7, 8, 9, and 10). Brücke (4) held the view that *retraction* of the villus squeezed out fluid from the central lacteal, while Graf Spee (7) thought that *lengthening* of the villus forced fluid into the deeper lymphatics. Several recent workers (8, 9, 10) incline to Brücke's view. Biederman (11) pointed out, long ago, the incompatibility of these views and emphasized the need for more careful study. The contradictory opinions as to the change in volume which occurs when the villus shortens depend, it would seem, on whether the individual observer has decided from his observations that the villi do or do not become thicker as they shorten.

Our observations indicate that the villi always thicken during retraction.

As this thickening often occurs very largely at the base the change in form is readily missed for the villi are so closely packed together that the bases are hidden from view. Only when the surface of the mucosa is folded so as to be convex do the villi fall apart so as to allow an unobstructed, profile view of their bases and shafts. Such a condition may be brought about by the use of negative pressure applied to the solution in the special absorption chamber. This will allow the gut, especially if it has been previously relaxed by the intravenous administration of atropine, to be bellied upward between the cross-wires of the grid. In favorable circumstances the villi may be observed to lie flat, and the changes in shape on retraction may be clearly observed, from the tip to the base. The shaft may not change perceptibly, but in many instances a definite thickening may be observed in this region (fig. 2, *b* and *b'*). The base, however, is always considerably thickened (fig. 2, *c*). The amount of bulging in this region appears to correspond to the degree of shortening. When the central lacteal is filled with a milky fluid it may be clearly seen to bulge at its base when the villus retracts, but when the villus regains its normal length the lacteal appears exactly as before. On several occasions single villi have been caused to retract repeatedly by stroking them lightly, but even after twenty to thirty successive retractions the central lacteal still remained filled. The contractions were not effective as an emptying mechanism.

When the rhythmical retractions of isolated villi, freshly removed from the living gut as described in the section on methods, are observed under the microscope the same illusion of shortening without change in diameter may appear when one pays attention only to the shaft of the villus; but here again the bulging of the base may be clearly seen (fig. 2, *a* and *a'*). Blood corpuscles are seen to shift back and forth in the vessels during such changes in shape, but none escape from the base, which is usually tightly closed off by a retraction of the epithelium that effectively prevents the passage of materials through the severed end of the villus. Fluid in the central lacteal may also be seen to shift during retraction but there is no evidence of escape from the severed base. Consequently we have to deal with a villus which apparently cannot change in volume, but which nevertheless can shorten in the normal manner with all the appearance of change in form which can be observed in the living animal. The evidence is therefore entirely opposed to the older concepts of villus activity as involving a pumping action of any type.

The fallacies of the villus-pump theory are further demonstrated by the complete absence of correlation between the degree of rhythmical activity of these organs and the rate or direction of passage of fluid, as studied by the aid of the special absorption-observation chamber, described above in the section on methods. The villi may be very active during absorption, but they may be equally active without transfer of fluid or even during

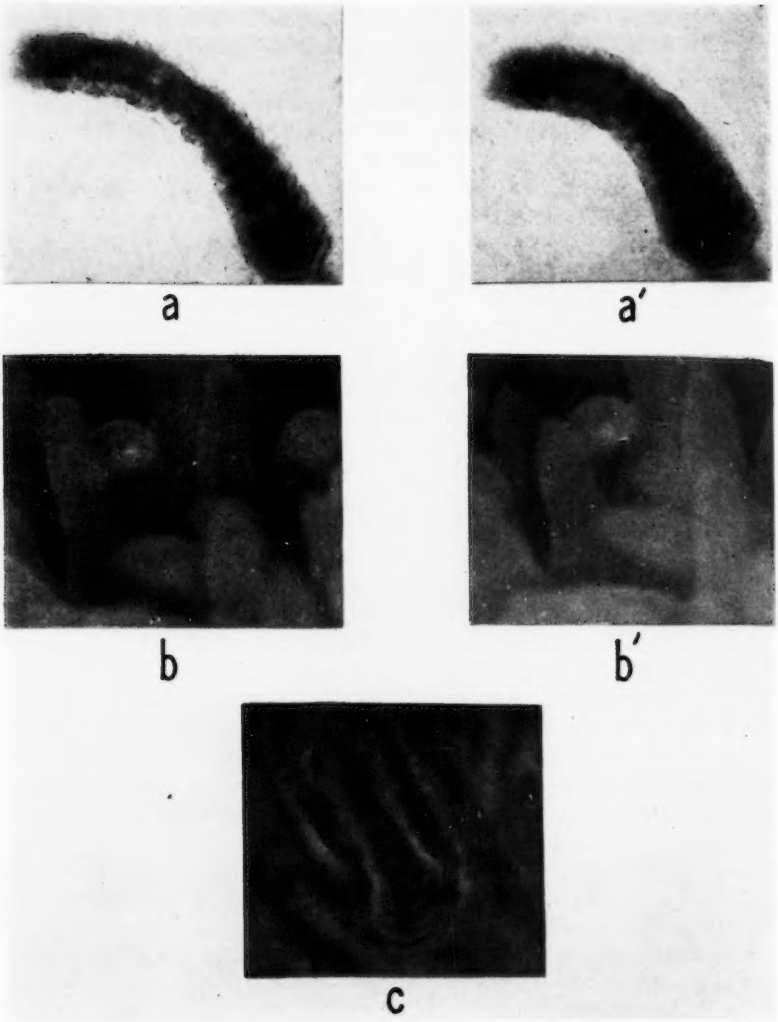

Fig. 2. An isolated villus is shown at *a* and at *a′* in the elongated and retracted positions respectively. Note the thickening of the villus on retraction, especially at the base but also along the shaft. Intact villi are shown at *b* in the elongated and at *b′* in the retracted positions. Thickening of the tips and shafts is evident in *b′*. Bulging of the base of an intact villus on moderate shortening is shown in *c*.

TABLE 1

The correlation of various appearances of the capillary circulation of the villi with villus movements and with the direction of flow of fluid through the gut wall

CIRCULATION			VILLI				FLUID TRANSFER
Artery	Vein	Open capillaries	Blood flow	Rhythmical movements	Shape	Color	
Constricted	Unobstructed, slender	Few	Sluggish, intermittent	++++	Slender	Pale, pink	Absorption rapid
Constricted	Engorged	Moderate number	Sluggish, intermittent	++++	Slender	Cherry	Absorption moderate
Moderately dilated	Unobstructed	Few	Moderate, almost continuous	++	Slender	Pink	Absorption moderate
Moderately dilated	Engorged	Moderate number	Slow, almost continuous	++++	Moderately swollen	Dusky	None; or slow secretion
Widely dilated	Unobstructed	Many	Rapid, continuous	None	Various	Bright red	None; or slow secretion or slow absorption (depending on degree of arterial dilatation)
Widely dilated	Engorged	Many, dilated	Slow, continuous	++++ or tonic	Swollen	Dusky	Secretion rapid

secretion, and conversely, absorption or secretion may be observed in the absence of any movement of the villi whatever. The observations are summarized in table 1.

The myogenic nature of villus retractions. The fact that isolated villi, freshly removed from the intestine of the living animal, can retract in a normal manner indicates conclusively that the smooth muscle elements exhibit an automatic type of behavior. All three types of villus retractions, the whipping, the tonic and the rhythmical, can be observed in these isolated specimens. The myogenic nature of the retractions, first suggested by King (3), is further evidenced by the fact that the rhythm of the retractions may be the same as that in the living animal. Retraction rates as high as twenty to thirty per minute have been observed, for short periods of time. Retractions at lower rates may continue for many minutes. It is therefore probable that the rhythmic retractions in the intact animal are not set off by nerve impulses: certainly the intervention of the submucous nervous system is not, as has been claimed, essential for villus activity.

Attempts to determine the optimum conditions for activity of isolated villi have given results which are far from conclusive. The activity is maintained for a longer time in blood serum than in any other medium so far tried. The villi are more active in Ringer's solution than in isotonic sodium chloride. They show no clear cut reaction to moderate changes in the calcium-potassium balance or in the pH of the medium. As might be expected the retractions persist longer in the presence of oxygen than in its absence and the contraction rate is higher at body temperature than at lower temperatures. In distilled water the villi cease to move and the epithelial cells swell up and finally degenerate into an amorphous mass which becomes separated from the basement membrane. The epithelium meets a similar fate when the villi are in salt solution, but the change comes on much more gradually. At pH 6.5 the villi continue to retract for a longer time and begin to show signs of epithelial degeneration much later than they do at pH 7.7. A definitely alkaline medium of any sort appears to inhibit the rhythmical retractions and causes the villi to contract tonically into compact, spherical masses.

It is possible that the difficulty of demonstrating definite reactions to moderate changes in the fluid environment has been due to the fact that the smooth muscle elements of the isolated villi are so well protected by the epithelial covering.

The question naturally arises as to what may be the natural stimulus to villus activity, but from the observations carried out so far we can only conclude that many types of simple physical and chemical changes in the environment of the villus may elicit a series of rhythmical contractions or whipping movements, or vary the rate or amplitude of movements already taking place. King, Arnold and Church (12) have shown that tying of

either the artery or vein of the mesentery will initiate rhythmic retractions of the intact villi. Our own observations, to be described, indicate that normally the conditions which are conducive to these movements have to do with a sluggish local circulation, whether this condition be due to arterial constriction or venous engorgement or both.

Interrelation of size, shape and movements of the villi, the appearance of the local blood circulation, and the direction and rate of transfer of fluid through the intestinal wall. Our analysis of the more than 50 experiments is summarized in table 1 and a description of the findings, upon which this classification of the observations is based, follows.

The first group of phenomena, that referred to in the table as correlated with a constricted villus artery and unobstructed collecting vein is characteristic of the resting, unstimulated mucosa. The gut of a fasting animal may be considered to be a resting organ, at least with respect to its mucosa. The walls of such an empty gut are folded tightly together, and the mucosal layer is pale and thin in contrast to the red, much swollen mucosa of an animal whose intestine is examined during the height of digestion. The villi are pale pink in color, long and thin, almost bloodless, and highly active in the performance of rhythmic retractions and whipping movements. The vein is thin and contains clumps of red corpuscles separated by clear plasma. At times no movement of the blood is seen; then, of a sudden, a bright red spot becomes visible at the tip of the villus. It enlarges and pulsates synchronously with the arterial pulsations of the animal, and a thin stream of blood flows rapidly through the few, mainly longitudinally disposed, capillaries, and on into the veins. Within a few seconds the flow ceases, to be resumed within several seconds to a minute. Retractions are more apt to occur during a prolonged cessation of flow than during a prolonged continuation of one of the periodic relaxations of the arteriole. When a retraction occurs the blood flow ceases at once, apparently as the result of compression; the capillaries and sometimes the veins are emptied, and the villus remains pale and bloodless until the relaxation allows the blood to return, either from the deeper veins alone, or from the artery also, if this should happen to be open. It is apparent in the resting mucosa that the arterioles which feed the villi are under a considerable degree of tonic constriction. Periods of complete closure of these vessels are succeeded, intermittently and very irregularly, by periods of partial relaxation. The central artery, situated near the axis of the villus, is not readily visible, although under ideal conditions it may be seen as a slender, pulsating strand. At the tip of the villus one sees a bright red point which is evidently an endothelial sinus arising from the end of the arteriole and from which the main capillaries of the organ radiate. This red spot disappears as the artery becomes completely closed, to reappear and to show the pulsatile increases in size, described above, when the artery opens again. The

proximal point at which the arterial lumen becomes occluded has not as yet been observed, but it is probably located near the base of the villus, or in the submucosa.

The maximum rate of fluid absorption and the greatest intensity of the absorbing force which, as has already been shown (1), may persist unchanged for many hours, are definitely associated with the particular type of sluggish, intermittent circulation described above as characteristic of the pale, resting mucosa. We may therefore conclude that the resting gut is an absorbing, rather than a secreting, organ; and it is to this resting condition that the mucosa tends more or less rapidly to return after any change that may be induced by local mechanical or chemical irritation or by various, not too drastic, forms of interference with the normal circulation of the gut.

As a matter of historical interest it should be noted that Graf Spee (7) observed the intermittent flow of blood in the vessels of the villi. He also noted the blanching reaction of a single villus which results from light mechanical stimulation. King and Arnold (3) have described several types of blanching reactions. More recent observers (8) who apparently encountered only those cases in which the villi exhibit a considerable degree of arterial dilatation or venous congestion have denied the possibility that rhythmical retractions may affect the state of the local circulation.

The second group of phenomena of table 1 is found in cases where the villus arterioles are also constricted but the veins are moderately engorged and the mucosa is somewhat bluish in color. The rate of absorption of fluid is slower, at any given intra-intestinal pressure, than in the case of a mucosa of the first type, and this retardation in rate is found to be due, as might be expected, to a less powerful absorbing force. The veins are always filled with blood and the retractions of the villi, which may be as frequent and as forceful as in the case of the first group, do not result in complete expulsion of blood from either the veins or the capillaries. This condition is associated, in our experience, either with an increase in tone of the outer gut musculature, such as may be produced by cold, or with a moderately elevated portal venous pressure (150 to 200 mm. water). It is well known that barbitalized animals often present the picture of engorged abdominal veins, and we have encountered all degrees of this condition in a number of our animals. Analysis of the condition is as yet incomplete, but we have noticed that any procedure which will lower the portal pressure will, often within a few seconds, restore the mucosa to the resting appearance, typical of the first group of the classification. The intravenous injection of 1 mgm. of atropine is usually followed at once by a fall of the portal pressure, in animals in which this pressure is elevated, and the volume recording lever gives evidence, within a minute or two, of an increase in the absorption rate and force. The drug is entirely without effect on either absorption of fluid or the appearance of the villi in animals which do not

present initial evidence of venous congestion. The inadequate respiration and resulting cyanosis of the animals has led us to employ artificial respiration, or better, to stimulate respiratory movements by the injection of air into the thorax. With the improved respiration there occurs a fall in the portal pressure, followed by the effects noted above. Such experiments indicate a definite relation between local capillary pressure and the force and rate of fluid absorption.

The remaining groups of the classification refer to conditions, often temporary, in which, by one or another of a variety of artificial procedures, the arteries of the villi are caused to dilate. One such procedure is mechanical stimulation, which, as is well known, produces an active hyperemia and calls forth a copious secretion of intestinal juice. The mechanical stimulation incident to the application of the special intestinal clamp provokes a marked reddening of the mucosa, and a rapid secretion of fluid. The volume recorder traces a steeply rising curve. In the case of an originally pale, resting gut the curve of secretion becomes progressively and rapidly less steep, finally tapering off to the horizontal, and within 10 or 15 minutes it turns downward and records decreasing volume. Within 15 to 30 minutes after the application of the clamp absorption begins and 5 to 10 minutes later the curve of absorption becomes a straight line, whose slope remains constant for hours, as described previously (1). The gut has returned very rapidly to its resting behavior and appearance as described above. Rhythmic movements of the villi, which had disappeared entirely during the stage of hyperemia, have returned. It is worthy of note that the resumption of these movements does not occur until some seconds or minutes after the local circulation has become sluggish and intermittent. In fact, as will be pointed out again, the rhythmical villus retractions are always associated with a sluggish flow of blood. An occasional, individual villus may often be seen in which the flow of blood continues for some time without interruption and this villus remains motionless in spite of the fact that it is surrounded on all sides by pale and active neighbors. It is only after its own arteriole has acquired a high degree of tone that the villus will be seen to retract.

Hyperemia of the normal gut, when produced as the result of local stimulation, begins to subside as soon as the irritant is removed, provided the gut has not been definitely injured by the irritating agent. In this connection it is worthy of note that the mucosae of certain animals, which have the chronic summer diarrheas so prevalent in dogs in this climate, remain hyperemic in spite of the most careful handling. The mucosa shows evidence of injury from the disease, for even light stroking with the tip of a wet sponge elicits bleeding; and secretion persists, with no evidence of the usual tendency of the mucosa to resume its normal or resting appearance and behavior. Hyperemia resulting from the operation of factors which

are remote from the mucosa may also persist in the absence of local stimulation. The "paralytic secretion" resulting from cutting the mesenteric vasoconstrictor nerves is a well known example of such cases. Paralysis of vasomotor tone, or the elevations of portal pressure noted above as resulting from the action of barbital, will produce hyperemias—active in the first case, passive in the second—recovery from which depends on restorative processes that are not within the jurisdiction of the local mucosal reactions.

In general the degree and duration of, as well as the extent of participation of the villi in, the response to irritation of the normal mucosa depend on the intensity and duration of the applied stimulus. There is, however, no very evident relation of the response to the kind of irritation. For example, solutions of bile salts, whole bile, duodenal contents, soap solutions, and hypertonic solutions such as 2 per cent sodium chloride, 10 per cent magnesium sulphate or 8 per cent glucose, produce reactions of hyperemia and tonic retractions of the villi which are indistinguishable from the reactions to mechanical irritation. In fact, the data suggest that saline cathartics probably act osmotically to irritate the mucosa, but that the resulting outpouring of fluid probably is not due so much to osmosis as to the results of the irritation.

If a pale, slender villus is touched near its base it retracts and becomes white and bloodless, but it soon relaxes to its original length. If the probe is pushed more vigorously against the base of the villus or down between the villi a number of these organs, in a radius of several millimeters, will retract very forcibly and will remain tonically retracted for some time. This type of retraction, together with the pitting of the mucosa which occurs in the region, is associated, as has been shown by King and Arnold (3), with contraction of the underlying muscularis mucosae. The area of tonically retracted and mushroom-shaped villi is at first blanched, as the result of compression of the local blood vessels. But the villi soon become pink again and they gradually elongate and resume their original appearance. A somewhat more violent stimulation results in the tonic retraction of a larger group of villi, which become white for an instant, then suddenly redden until they appear almost hemorrhagic. Microscopic examination shows that numerous fresh capillaries have opened up. These, which are not seen in the normal villus, run mainly in a transverse direction, near the tip, and connect the main capillaries which run longitudinally. The flow of blood in these fiery red villi is extremely rapid at first, but after a minute or more the color becomes distinctly blue and the flow of blood is much less rapid and practically may cease. It is probable that the tonic contraction of the muscularis mucosae is responsible for this obstruction to the venous outflow, of which the local cyanosis gives unmistakable evidence. The venous obstruction, together with the no less obvious ar-

terial dilatation, must produce a high capillary pressure—a pressure which, indeed, must approach that of the arteriole itself, so slow is the flow of blood. Such unmistakable evidence of the high capillary pressure brought about by the combined reactions of local blood vessels and of the villus and submucous muscular elements is sufficient to explain the source of the fluid which is secreted so copiously as the result of irritation. The capillary filtrate may be seen, under these conditions, to distend the central lacteal and to cause a rapid and pronounced increase in the size of the villus, which presents a smooth, rounded surface and more nearly spherical form. It is difficult to avoid the conclusion that the pressure within such swollen villi is sufficient, of itself, to explain the transfer of fluid through the epithelial membrane and into the lumen of the gut. The failure of the fluid, which accumulates within the villi, to drain entirely away by the lymphatic route may perhaps be due to a compression of the larger lymphatic trunks lying in the submucosa.

That fluid, under pressure, may pass freely from the central lacteal to the lumen of the gut is evidenced by the appearance of milky fluid in the lumen following vigorous mechanical stimulation of the mucosa of an animal which has recently (within 12 hours) been fed on a meal of milk or cream. The disappearance of this milky lymph from the central lacteal and its gradual replacement by a clear fluid may be noted in such animals, thus confirming King, Arnold and Church (12).

The time required for restoration of the normal resting appearance of the mucosa after removal of an irritating agent depends on the degree of alteration of the mucosa which has been produced, and this in turn depends on the intensity and duration of action of the applied stimulus, and may vary from a few seconds to several hours. The recovery process is initiated by a gradual relaxation of the tone of the muscularis mucosae and the elongation of the villi thus made possible. The flow of blood becomes freer and the villi become red in color. With a gradual increase in vascular tone the capillaries disappear and the pale color of the resting organ is approached. The process of attenuation of the turgid, swollen villus starts at the tip and progresses toward the base. At one stage the basal half of the organ may be twice as thick as the now normally slender distal half and the line of demarcation between these two portions may be quite sharp. We may tentatively assume that the tissue elasticity near the tip is greater than that near the base; in fact such a condition will explain the elongation of the villus after a retraction.

It is noteworthy that the gut, when examined in the early stages of digestion, presents a much thickened, water-logged mucosa. There is active hyperemia and there may be some degree of tonic retraction of the villi, but this is not marked. The villi are swollen as reported by King (12). The picture is identical with that presented by an originally resting gut

which has been subjected to long-continued, mild, local stimulation. Secretion of fluid occurs in both cases and an hour to three hours may elapse before the mucous membranes will have given up their excess of fluid and resumed the pale, thin-walled appearance of the resting gut. Absorption begins only after the process of recovery has progressed nearly to completion. Consequently we can assume that the excess of fluid in the mucosa and submucosa of the digesting gut does not represent recently absorbed water but rather a fluid which has filtered out from the blood vessels. The absorption of fluid from the digesting material apparently cannot commence until the processes of digestion and of dilution by secreted fluid have rendered the material isotonic and otherwise non-irritating. Only then can the digestive hyperemia subside and allow, first the absorption of the excess of fluid from the tissue spaces and eventually the absorption of fluid from the lumen. That absorption of dissolved substances can occur against a flow of secretion of fluid can scarcely be denied—a similar phenomenon occurs in simple physical systems—and one is not unduly surprised to find that the central lacteals are often filled with a white chyle even in the early stages of digestion, when secretion of fluid is still moderately rapid.

It is probable that any condition which increases the tone of the outer gut musculature will cause a higher degree of venous obstruction and passive hyperemia than can occur through agencies acting on the muscularis mucosae alone. Thus reactions to pilocarpine, given intravenously, occur quickly and the mucosa becomes so thickly covered with mucus that it has not as yet been possible to observe whether arterial dilatation is produced by this drug, but the deep cyanosis gives evidence of marked venous engorgement.

Adrenaline in the large doses of 2 to 10 cc. of a 1:10,000 solution, injected slowly, is known (11) to cause a marked rise in portal pressure, as well as an increased flow of blood through the gut. The combination of active and passive hyperemia so produced results in a profuse secretion of fluid, which, however, ceases as the blood pressure returns to normal.

Hypertonic sodium chloride solution when injected intravenously produces much the same type of effects as when it is placed directly on the mucosa, but the reaction is far less intense, perhaps as the result of dilution in the blood stream. A blanching followed by intense hyperemia, profuse secretion and the appearance of a tonic retraction of the villi occur. The effect may be explained on the basis of irritation, like that due to local application of strong solutions. The increase in blood volume by removal of water from other tissues may play a part in the secretion.

The injection of large amounts (100 to 300 cc.) of isotonic saline is followed by a gradually increasing secretion of fluid. The circulation becomes more rapid, perhaps mainly due to the lowered viscosity of the blood. There is no reaction of hyperemia or of tonic retraction of the villi as occurs

after the injection of hypertonic solutions. The large amounts of fluid are known to cause a marked rise in the portal pressure, hence we may assume that the congestion will produce an increase in the capillary pressure in the villi, which in fact become very much swollen. There is a profuse intestinal secretion which may well represent a mechanism for the regulation of blood volume and concentration which should be given added attention in physiological and clinical investigations.

SUMMARY

1. A special absorption chamber permits observation of the intestinal villi and their circulation during experiments in which the direction and rate of fluid transfer through the wall of an isolated loop of gut are also recorded.

2. Lack of correlation between the motor activity of the villi and the rate or direction of passage of fluid, together with observations indicating that the villi do not change in volume as they shorten and that the central lacteal is not emptied by this mechanism, argue against the validity of the villus-pump theory of absorption.

3. Isolated villi, freshly cut from the mucosa, exhibit the same type and rhythm of movement as do the intact organs. It is concluded that the retractions are myogenic rather than neurogenic in character.

4. Rhythmic retractions of intact villi are correlated with a sluggish flow of blood through the local capillaries, while a rapid flow is always associated with complete inactivity of the villi. Changes in activity seem, therefore, to depend on simple chemical factors associated with exchanges between the smooth muscle cells of the villi and the blood.

5. Absorption of fluid occurs only when the villi are slender and pale and the local flow is intermittent and sluggish. Such an appearance suggests that the capillary pressure and hydrostatic pressure of tissue fluids of the villi are low.

6. Secretion of fluid occurs only when the converse picture of hyperemia (active or passive, or more often both) is present. The villi are tensely swollen and the mucosa is thickened and water-logged. Capillary and tissue fluid pressures appear to be high.

7. The resting gut is an absorbing organ. Local irritation, whether mechanical, chemical or osmotic (saline cathartics or other hypertonic solutions) results in hyperemia of the mucosa, swelling of the villi and tonic retractions that are effective in impeding the venous and possibly the lymphatic drainage, and causes profuse secretion of fluid.

8. It is probable that secretion, as well as absorption, of fluid is a physical process, and that transfer of fluid through the gut wall is regulated primarily by vascular reactions, both local and general.

We wish to take this opportunity to express our gratitude for valuable advice and encouragement given us by Dr. C. E. King of this department, whose pioneer work in the direct observation of villus movements provided the starting point for the present study.

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CHANGES OF THE CARBOHYDRATE METABOLISM OF THE HEART FOLLOWING CORONARY OCCLUSION[1]

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The changes in the carbohydrate metabolism of the heart deprived of its blood supply have hitherto received little attention. The perfused beating heart maintains its glycogen content as long as sufficient oxygen is supplied (1), while lack of oxygen results in a rapid depletion of the glycogen of the myocardium (2). The normal heart has been shown to remove lactic acid and glucose from the blood, and presumably convert them to glycogen (3, 4, 5). The conversion of these precursors of glycogen requires energy which probably arises from oxidations. It was therefore thought of interest to study the chemical changes of the heart under conditions of diminished oxidations resulting from experimental coronary occlusion.

METHODS. The thirty-four dogs in this study included three which were under the influence of phlorhizin: three had survived moderate doses of adrenalin: seven were depancreatized at least forty-eight hours before the present experiments: ten were subjected to bouts of acute alcoholism twenty-four hours before the heart studies were made: and eleven were normal animals. Since each animal served as its own control and no changes were found which might be attributed to the previous regime, the animals were not differentiated in the following tables.

The animals were anesthetized with sodium amytal, and then under artificial respiration the sternum was split thus exposing the heart. A ligature was passed around a branch of the left anterior descending coronary artery. After the artery was ligated an area of varying size became purple in color, and in some instances ceased to contract. The ligature was maintained for periods varying from five minutes to one hour.

In the first of two groups of experiments observations were obtained on twenty-three animals. At the termination of the experiment the infarcted area and a portion of the normal myocardium were excised. These were plunged into chilled alcohol directly, or first into liquid air and then chilled

[1] A preliminary report of this work was published in the Proc. Conn. State Med. Soc., 1932, 115–122.

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alcohol. The tissues were triturated and remained in alcohol in the ice-chest for 18 hours. The alcohol was evaporated from an aliquot of the alcoholic filtrate and the remaining lactic acid was determined by the method of Friedemann, Cotonio and Shaffer (7). The soluble carbohydrates were estimated according to the fermentation method suggested by S. R. Benedict (8). The glycogen of the remaining tissue was analyzed by a modification of Pflüger's method (9). The accuracy of the above methods was estimated from the fact that duplicate determinations on adjacent portions of normal myocardium gave the following results: Lactic acid ± 5 mgm. per cent, glycogen ± 10 mgm. per cent, and soluble carbohydrates ± 14 mgm. per cent.

In the second group of experiments observations were made on thirteen animals. Samples of blood were drawn from the femoral artery and coro-

TABLE 1

The changes in carbohydrates of the occluded area of the myocardium

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
GLYCOGEN		LACTIC ACID			SOLUBLE CARBOHYDRATES			TOTAL BREAKDOWN PRODUCTS		
		Decreased	No change	Increased	Decreased	No change	Increased	Decreased	No change	Increased
Decreased	8	1	2	5	2	2	4	1	1	6
No change	9	3	2	4	2	2	2	1	1	4
Increased	1	0	0	1	0	0	1	0	0	1
Not done		0	0	5	0	2	2	0	0	4
Total	18	4	4	15	4	6	9	2	2	15

nary vein before and after the coronary occlusion and analyzed for lactic acid and glucose.

RESULTS. The relationships between the changes in glycogen, lactic acid and soluble carbohydrates are presented in table 1. Since there may be differences in the glycogen concentration of various portions of the heart, we have called changes of 100 mgm. per cent significant despite the fact that determinations on adjacent portions of normal myocardium agreed within 10 mgm. per cent. When differences of 20 mgm. per cent were taken as significant, the general picture presented was not altered.

The eighteen observations obtained on the glycogen concentration of portions of normal and infarcted myocardium are presented in column 2. Nine showed appreciable differences, and in 8 of these the glycogen concentration was lower in the infarcted area. This portion of the heart contained an average of 260 mgm. per cent less glycogen than did the normal area.

The changes in lactic acid and soluble carbohydrates in each group of

experiments listed in column 2 are noted in columns 3 to 11. In the 8 observations in which the glycogen of the infarcted area was reduced, the lactic acid rose 5 times and the soluble carbohydrates increased 4 times. The sum total of the breakdown products of glycogen, namely, lactic acid plus soluble carbohydrates, increased in 6 instances and decreased only once. In the 9 experiments in which the glycogen content of the infarct was equal to that of the normal heart, the changes in lactic acid and soluble

TABLE 2

Lactic acid of femoral artery and coronary vein before and after occlusion of the coronary artery

DATE	BEFORE OCCLUSION		AFTER OCCLUSION		LACTIC ACID BALANCE	
	Femoral artery	Coronary vein	Femoral artery	Coronary vein	Before occlusion	After occlusion
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
1931						
12/22	29	25	14	26	+4	−12
1932						
5/18	22	18	24	29	+4	−5
7/7	32	18	32	21	+14	+11
7/20	21	13	23	24	+8	−1
7/25	48	18	40	40	+30	0
7/28	18	14	19	32	+4	−13
8/3*	29	18	27	35	+11	−8
10/12*	78	84	108	128	−6	−20
12/9	32	38	44	50	−6	−6
1933						
12/8	25	24	33	30	+1	+3
1934						
1/4	34	32	45	43	+2	+2
1/10	30	29	41	43	+1	−2
Average					+8	−6

* Histological sections of the infarcted area revealed no evidence of morphologic change.

carbohydrates were quite variable. Only in 6 of these 9 experiments were both lactic acid and soluble carbohydrates analyzed. In 4 cases there was an increase in the total breakdown products of glycogen, and only once was there a decrease. The source of these breakdown products might still be glycogen without being demonstrable by our methods, if the glycogen changes were less than 100 mgm. per cent. In 5 experiments no glycogen determinations were made and the lactic acid of the infarct rose each time

and the soluble carbohydrates rose twice. In 4 of these experiments both lactic acid and soluble carbohydrates were analyzed and the infarcted area contained a higher concentration of these substances than did the normal tissue in each case.

The results obtained on blood lactic acid before and after coronary occlusion are presented in table 2. Before occlusion there were significant differences between the concentration of lactic acid in the femoral artery and coronary vein in 9 experiments. In 7 of these the heart was removing lactic acid from the blood, the average amount removed for the entire series being 8 mgm. per cent. After occlusion the heart liberated lactic acid in 6 of the 8 experiments in which significant differences were noted and the average liberation was 6 mgm. per cent. A further analysis of these data shows that in 4 instances the heart removed lactic acid before occlusion but added it to the blood after the coronary artery was tied. In 5 other experiments the heart was less able to remove lactic acid from the blood after occlusion as indicated by the change in the lactic acid balance of 3 mgm. per cent or more.

A series of 7 observations was made on the glucose concentration of samples from the femoral artery and coronary vein. The heart was found to remove glucose from the blood both before and after occlusion in most instances. These data are not presented in order to save space.

Discussion. The volume of oxygen supplied to muscular tissue has long been known to be of paramount importance. In the presence of oxygen the lactic acid concentration in the tissues remains low, and the glycogen high. In the absence of oxygen, or when the tension of this gas is diminished lactic acid tends to accumulate at the expense of glycogen. In skeletal muscle the concentration of lactic acid has been shown to rise to 300 mgm. per cent before the muscle ceases contracting. If, however, oxygen is admitted before total paralysis occurs, the lactic acid disappears, glycogen reappears and the contractile power is restored once more. In the case of heart muscle a similar mechanism operates causing lactic acid accumulation when the oxygen supply fails. This specialized muscle, however, is much more sensitive to lactic acid since a concentration of only 70 mgm. per cent will cause fatigue and cessation of contractile response (10).

In the present experiments the acute ischemia of a portion of the myocardium resulted in a marked fall in the glycogen content of the infarcted area. Other observations yielded evidence of the fate of the lost glycogen. There was a local increase in lactic acid concentration, which in some instances reached levels incompatible with further contraction.[2] In addition there was an increase in soluble carbohydrate products intermediate be-

[2] While preparing this paper for publication we learned that Grayzel, Tennant, Stringer and Sutherland (11) have obtained similar observations on the glycogen and lactic acid changes of the heart during coronary occlusion.

tween glycogen and lactic acid. In this area of diminished oxygen tension the energy for contraction would necessarily be produced in a larger measure from anaerobic sources. The present experiments indicate that the principal source of this energy was an increase of glycolysis, an anaerobic reaction.

Though the normal heart usually absorbs lactic acid from the blood, after occlusion the heart was found to pour lactic acid into the blood stream. The lactic acid may have arisen in the affected areas in which the oxygen tension was diminished. This reduced oxygen supply of the infarcted myocardium prevents the reconversion of lactic acid to glycogen which normally takes place at the expense of oxidations. If as a result of the trauma the efficiency of the uninfarcted area is also lessened, then this portion of the cardiac musculature, requiring a greater amount of energy to perform its functions, could obtain it from an anaerobic source, namely, the breakdown of glycogen to lactic acid. To such changes may be imputed the reversal of the action of the heart on the lactic acid of the blood. The data on glucose, though not extensive, reveal that that substance may still be absorbed by the heart after occlusion of a branch of the coronary artery. In the uninfarcted area this glucose may either be converted to glycogen, or oxidized to supply energy; while in the infarcted portion of the myocardium it may give rise to lactic acid.

Not only objective, but also subjective changes may be produced by ischemia of muscle. It has been shown that with a diminution of blood supply to skeletal muscle which gives rise to an increased concentration of lactic acid, there also appears the subjective symptom of pain (12). The present study presents evidence of the increased concentration of lactic acid of the infarcted area, and since pain is an integral part of the syndrome of coronary occlusion, it is suggested that a relationship may exist between the rise in lactic acid of the infarcted area and the production of anginal pain.

SUMMARY

Observations were made on 34 dogs during experimental coronary occlusion. The infarcted area lost appreciable quantities of its glycogen which appeared in part as increased amounts of soluble carbohydrates and lactic acid. In most cases before occlusion the heart removed lactic acid from the blood stream. After the coronary artery was ligated the heart usually added lactic acid to the blood stream. The increased lactic acid production by the heart was probably due to a diminution of the O_2 supplied to the cardiac tissues. Glucose was absorbed from the blood both before and after the coronary occlusion. It is suggested that the accumulation of metabolites in the infarcted area may be related to the subjective pain experienced during coronary occlusion.

We are glad to express our indebtedness to the staff of the Department of Pathology for the study of the histological sections mentioned above.

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THE SIGNIFICANCE OF FREQUENCY, NUMBER OF IMPULSES AND FIBER SIZE IN VASOMOTOR RESPONSES TO VAGUS AND DEPRESSOR NERVE STIMULATION IN THE RABBIT

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On the supposition that the relation between stimulation of the depressor nerve of the rabbit and the consequent lowering of blood pressure might be as simple as in any reflex action, we have attempted to find what that relationship is. In a previous paper we have discussed several of the complications; that the number of fibers in the nerve varies widely, that the blood pressure effect per fiber number varies also, that both pressor and other afferent fibers may be present, and that a variable number of presumably motor fibers complicates the picture. We discuss here various aspects of the effects of stimulation in certain depressor nerves where the only reflex effect detectable under our experimental conditions was a lowering of blood pressure, on the assumption that in any rabbit, under constant conditions, the peripheral effect is a fairly direct measure of the effect produced on the center by afferent stimulation. This seems to be true when the number and frequency of stimuli are varied over such a range that the form of the curves of blood pressure lowering is kept constant. Certain observations are recorded on pressor and depressor fibers in the vagus. The technic has been described in a previous paper (O'Leary, Heinbecker and Bishop, 1934).

Form of the response. The rate of stimulation required to obtain the maximal effect in lightly etherized rabbits varies in different preparations between 40 and 100 per second. With a constant stimulus (as to strength, frequency and duration) repeated at intervals of several minutes, recovery from each depression takes place rather promptly, but to a falling base line, which can be interpreted as a failure of complete recovery. There seems to be an initial phase and a more prolonged phase of recovery of different orders of duration. With such a series of constant stimuli, how-

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ever, the *percentage* lowering referred to the pressure just before stimulation as a base line, is significantly constant. After such stimulation the pressure may remain at its lower level for over half an hour without full recovery. Strong stimulation of the vagus or of the saphenous causing a rise of pressure above normal, causes a prompt return to the initial or higher level, and this level is maintained for long periods or until further stimulation. If anesthesia is increased, the level falls similarly, but the percentage fall on stimulation is now reduced. If anesthesia is decreased, the base line becomes irregular, frequently showing slow periodic fluctuations, which can often be initiated by stimulation when they do not appear without. These are of lower frequency than either heart or respiratory rate.

These persistent changes of level due to stimulation, imitating only in certain respects the effects of anesthetic, are believed to be due to accumulations, presumably in the circulation as a whole, of the effects of stimulation, but we do not know whether the hormone-like action is central or peripheral. From the fact that the center still gives the same percentage effect as measured by peripheral response, and that the curve of response is of constant form, we infer that there has been merely a change of tone of the peripheral vessels, rather than a failure of complete recovery of the center.[2]

The first depression detectable takes place only after a latent period of two or more seconds. In typical records from nerves presumably without pressor fibers, the pressure falls nearly linearly at first, then flattens out more gradually to its new level, often showing a second step-like lowering after an initial minimum. The level may then remain constant until cessation of stimulation. In a previous paper (Bishop, Heinbecker and O'Leary, 1933) the second step has been assigned to vasodilator action via the dorsal roots, since in the perfused sympathectomized limb this delayed dilatation is the only one demonstrable. When the rate and duration of stimulation are varied, but within the latent period, that is, when stimulation is stopped before the response begins, the lowering of pressure is very closely proportional to the total number of stimuli applied at constant strength, between 16 and 100 per second. (Rosenblueth has also found nearly a linear relationship. Personal communication.) This is approximately true even if stimulation is continued during the fall of pressure, but is not true if stimulation extends beyond this. The complication is presumably that stimuli occurring after the maximal (central) effect of previous stimuli

[2] In case this finding be interpreted in the light of the current enthusiasm for central hormones, we might point out that if this be action of a substance responsible for typical nervous action, it would seem to have slopped over, either in space or in time, from its proper and typical locus of activity. That to be sure is something a hormone might be capable of.

has been attained, tend rather to maintain the activity than to increase it. If longer durations of stimulation are applied, however, the total area between the depressed curve and the original level is proportional to the number of impulses. This suggests a summation of central effect that is a linear function of the number of impulses, which is perhaps another aspect of Bayliss' observation that the reflex is non-fatigable. When pressor fibers are present, as indicated either by an initial rise of pressure on weak stimulation, or by a partial return after an initial fall, this simple relationship is naturally disturbed, indicating that pressor and depressor impulses do not summate in the same simple manner. When an initial pressor response is present, its latent period is much shorter than that for a pure depression. The latent period of the latter does not vary materially with strength of stimulus.

If frequency only is varied, and stimulation continues to a maximum effect, quite as when the total number of impulses is varied, but within the latent period, a nearly linear function is obtained, but with the larger range of depression here possible, the effect can be seen to be not quite proportional to the frequency. The more stimuli applied per unit time, the less the effect per stimulus. That the effect is so nearly linear is probably associated with the fact that the amount of depression obtainable by even maximal stimulation of one depressor nerve is not the maximum of which the animal is capable, and one is thus working along the nearly linear limb of an actually exponential or other non-linear curve. Even then, the final result might be only the smoothed average of a large number of variables. Except for our inability to discover from our limited data what the form of the curve really is, these findings parallel Rosenblueth's (1934) on heart rate, and are convenient as an approximate measure of the amount of stimulation received by the center, when different numbers and sizes of afferent fibers are activated.

Depressor action per fiber for fibers of different size. With short condenser charges as stimuli (0.0005 mf. usually gives a threshold at 3 to 4.5 volts, with one-half the current wasted in the bridge stimulus circuit), the maximal effect obtainable from stimulation of one depressor is usually reached at 2.5 to 3.5 times the threshold for first effect. In preparations where the threshold of the action current and the threshold for depression of blood pressure were the same, we have examined the relation between number of fibers active, size of fiber, and response, at different rates and durations of stimulation. The curve of percentage lowering of blood pressure plotted against strength of stimulus resembles that of action current area plotted against stimulus rather closely, and since, with constant voltage, increasing the number of impulses delivered to the center increases the effect nearly proportionally, a summation at least as effective may be inferred for the impulses from fibers of different size. This would mean that the amount

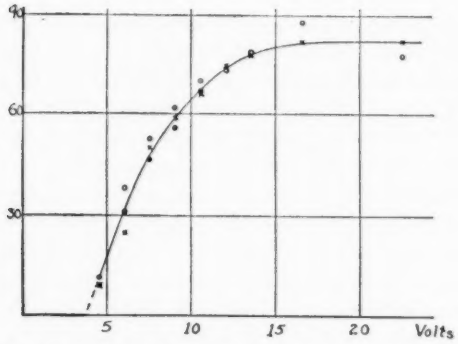

Fig. 1. Plot of action potential area (crosses) against strength of stimulus for rabbit depressor nerve. Circles, similar plot of per cent reduction in blood pressure coincidentally recorded; long durations of stimulation, on an arbitrary scale of y axis that allows comparison of form of 2 curves. Solid circles, blood pressure curve corrected for non-linear summation of stimuli, according to curves of figure 3. See text for details.

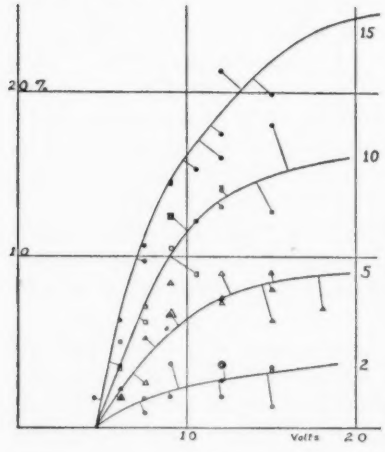

Fig. 2

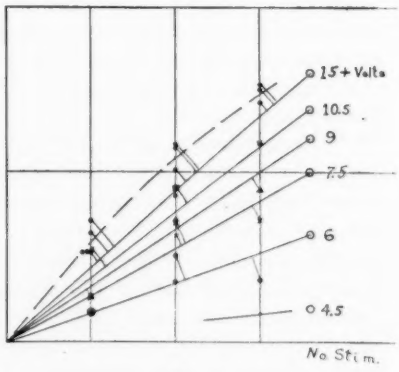

Fig. 3

Fig. 2. Plots of blood pressure per cent reduction against strength of stimulation for different numbers of stimuli. Figures to left of curves are revolutions of contact wheel, 2/second, 20 stimuli per revolution. The greater the number of stimuli, the less the effect per stimulus.

Fig. 3. Per cent reduction of blood pressure against number of stimuli for different voltages of stimulation. The straight lines through the origin should be the loci of the points if effect were proportional to number of stimuli for a given strength. The upper dash curve is the actual locus of such values at maximal stimulation strength, and the deviation from the straight line is a measure of the correction to be applied to convert per cent reduction of blood pressure into effect on the central nervous system.

of *central effect* per fiber varied as the action current area of the fiber, provided all the fibers which produced a voltage also depressed blood pressure. Unfortunately other fibers may be present, even though not detectable.

In figure 1, x is plotted the action potential against strength of stimulus up to the maximum of blood pressure effect for stimulation of long duration, from a nerve without obvious pressor activity. The per cent lowering of blood pressure is also plotted, ○, against the same stimuli, recorded coincidentally with the action potentials, on a scale that makes the two curves readily comparable.

The curve of blood pressure lowering requires a correction for comparison with the curve of action currents. Lowering of blood pressure is not exactly a linear function of the amount of stimulation in terms of number of stimuli of equal strength. How nearly linear this is may be determined experimentally. The greater the amount of stimulation (number of equal stimuli), the less the effect per stimulus. In figure 2 are plotted, for the same nerve as figure 1, the amounts of blood pressure lowering (per cent) for different numbers of stimuli at different strengths, each curve representing a plot of blood pressure against voltage. The values scatter more widely for the weaker effects, but the increase in any ordinate is less than proportional to the increase in number of stimuli. In figure 3 these data are replotted as depression against number of stimuli at different voltages. The points, ○, at the right of this figure are the maximal values from figure 1 for long durations of stimulation, plotted over the value for number of stimuli, after which these maxima are attained. The adjacent figures are the corresponding voltages. Straight lines through these and the origin should be the loci of the data plotted if depression were proportional to the number of stimuli, and the scattered points representing the data are joined to their appropriate lines. For weak stimulation the points are too scattering to discriminate, but for the higher voltages most of the points fall definitely above the lines, indicating a concavity toward the x axis; that is, with greater number of stimuli, the effect per stimulus falls off along a curve which deviates from the linear as indicated in the upper dash curve of figure 3. Using these values to convert the blood pressure lowering values of figure 1 to the corresponding amounts of stimulation which the vasomotor centers presumably received to produce them, and replotting on a scale that leaves the point for 14 volts stimulus unchanged, ●, it is obvious that this is at least the form of the correction required to bring the curves of action current area and central effect into coincidence.

This experiment gives the best agreement of this kind that we have obtained, and it also involves the most complete set of data on an animal whose blood pressure level was relatively steady. Other experiments indicate the same relationship, but less conclusively. All agree in the form of the curve of blood pressure against voltage, showing slight curvature at

the start, and a falling off, similar to an exponential, rather than an S-curve such as the plot of the integral of fiber number against fiber size. The curves of action potential areas have the same general form as the curves for blood pressure, and the same limits of stimulus strength. A deviation where it exists could be assigned to the responses of fibers demonstrably present in some nerves which do not cause lowering of blood pressure, provided it be further assumed that their distribution with respect to threshold (size) were not the same as that of the depressor fibers. This argument to be sure could be reversed, and the apparent agreement, between action potential area and central effect, be assigned to the appropriate distribution of non-depressor fibers. Such pitfalls have indeed been encountered in biological experimentation.

Insofar as action current area varies as the square of the diameter, which seems to give a fair fit in this range, (O'Leary, Heinbecker and Bishop, 1934) the central effect per impulse may be proportional to the cross-sectional area of the fiber.

Antagonism between pressor and depressor effects. When a mixed nerve is stimulated, various complications of pressor and depressor effects may be obtained, depending on strength and frequency of stimulation. One may obtain a pure rise, a pure fall, a rise, followed by a fall during or after stimulation, and a similar fall followed by a rise. A number of workers have found that at constant strength of stimulation, a low frequency, 4 to 20 per second, produces a fall, and a higher frequency a rise of blood pressure. Gruber (1917) has summarized and extended this work, and to other antagonistic actions than vasomotor effects, and has raised the question whether such reversals of action were due to a different effectiveness of frequency alone on the centers involved, the same fibers being capable of producing either, or whether different fibers were involved for opposite effects. This is a fundamental problem of central action, involving the specificity of nervous pathways, and has been touched upon more recently in a slightly different form by Blair and Erlanger (1933), if we understand them correctly, in a reconsideration of the significance of fiber specificity. In many depressor nerves, a pure depressor effect is obtained at any rate or strength of stimulation, while in all rabbit vagus nerves a strength of stimulus may be found such that an increase of rate will convert a depression into a rise of blood pressure. Since these two nerves have a common origin and presumably act on the same centers, they would seem to offer a satisfactory means of testing this phenomenon in further detail.

The pressor effects of the vagus vary more or less parallel to the depressor effects of the depressor nerve, that is, when a depressor gives a marked fall of pressure, the vagus is apt to give a marked rise on medium to strong stimulation, and vice versa, when the depressor nerve action is poor the vagus tends toward depressor effects. Further, a poor depressor nerve action

can be correlated with a smaller number of fibers in the nerve. These facts lead us to believe that the same purely depressor fibers which generally pass through the depressor nerve, can sometimes pass through the main vagus trunk. That is, some of the smaller vagus fibers are exclusively depressor fibers, without the ability to produce pressor effects. Likewise, large pressor fibers of low threshold when present in the depressor produce a pressor effect at any frequency, until at higher strengths of stimulus their effects are counteracted by those of depressor fibers. Further, some vagi, stimulated near threshold, produce only a depressor effect at any frequency, (fig. 6) and these threshold fibers are different in size from those characteristic of the depressor nerve (15 mu as against 5 mu or under). It is only in the range where both pressor and depressor fibers are presumably stimulated that a reversal of effect takes place with change of frequency. For a theory of a variable effect from the same fiber stimulated at variable frequency, one would then have to assume three categories of fibers in the vagus, pure pressor, pure depressor, and ambivalent. In view of the smoothness with which pressor and depressor effects may add, this seems at least to be a superfluous complication.

However, there is a more serious argument against what may be termed a resonance explanation. Afferent end organs in general appear to convey their message to the centers in terms of frequency and numbers of stimuli delivered to their nerve fibers, and only thus. If a vasomotor afferent end organ were weakly stimulated, on the resonance theory, one would expect a few responses at low frequency to produce a depressor effect, and if strongly stimulated, more impulses at higher frequency should produce a pressor effect; but as the frequency fell off, when the effects of a stimulus died away, the initial pressor effect of strong stimulation should be counteracted by the later depressor influence of the same stimulus, and possibly before the periphery with its long latent period would have time to decide which way to act. Further, a given stimulus would surely affect different afferent endings differently, so that some fibers would in general respond slowly, and thus oppose others responding more rapidly. Such a mode of action as the resonance theory connotes does not appear suitable as a mechanism for the mediation of two opposing activities.

On the other hand, a simple summation of the effects of two opposing mechanisms does not explain the results without subsidiary assumptions. Both effects may be practically non-fatigable, as indicated in figure 4, A and C; their latent periods are not widely different; and yet B in the same figure indicates that they add up to different amounts at different times, while F and G of figure 6 show that their order of occurrence can be reversed merely by increasing strength of stimulus, i.e., number of fibers active. Comparison of A, C and G of figure 7 shows that this reversal is not brought about by addition of numbers of *depressor* fibers, and the fact

that on weak stimulation the vagus gives predominantly depressor effects at any frequency indicates that strong stimuli which bring in pressor effects must activate predominantly pressor fibers. When complex effects follow vagus stimulation, the higher the frequency, not only the less is the depression, but the earlier is it reversed during stimulation.

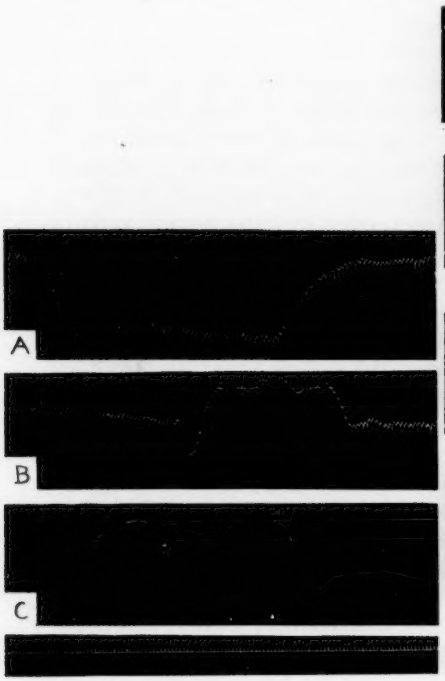

Fig. 4

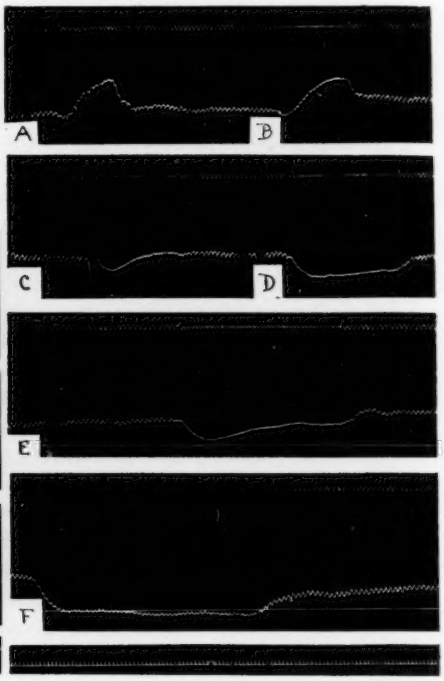

Fig. 5

Fig. 4. Vagus nerve, induction coil makes and breaks, 50 each/second. A, B and C, weak, medium and strong stimulation. (Artery clotting in C.) Make shocks may not have stimulated in A. Partial and complete reversal, in B and C, of almost pure depressor effect in A. Time in seconds, 120–130 mm. blood pressure, 30 mm. fall in A.

Fig. 5. Vagus nerve, A, B, C and D, decreasing strengths of induction coil makes and breaks 50 each/second. E, 10.5 volts condenser charges, 48/second, slightly more effective than D. F, 10.5 volts, 24/second, apparently pure depressor effect.

One may view the vagus as a mixed nerve from which a variable number of its smaller depressor fibers have been segregated into the depressor branch, leaving a majority of the small vasomotors, pressor fibers; and having as a majority of its larger vasomotors, depressor fibers. The rationale of the

effect of frequency when both groups of fibers are activated may be that the depressor center responds to an optimal frequency, above which the effect per impulse falls off rapidly, which is lower than the optimum for the pressor center. The stronger the stimulus, the vagus being what it is, the greater the proportion of the total fibers stimulated will be pressors, and the faster the stimulation, the less relatively the depressor effect produced,

Fig. 6. Vagus nerve, A, 4.5 volts condenser charges, 32/second. B, same, 96/second high frequency does not give reversal of depressor effect near threshold. C, 9 volts, 32/second, slight rise after fall, but less fall than with weaker stimulation of same frequency, i.e., pressor fibers stimulated only by stronger stimulus act antagonistically to depressors. D, 10.5 volts, 64/second. E, 10.5 volts, 96/second. The sum of pressor and depressor effects at higher voltage about equals that of depressors alone at lower, (B), but a partial rise follows the fall. F, 22 volts, 96/second. G, 45 volts, 96/second. Pure depressor effect of B, changing through E and F, a depression followed by a partial rise, to complete reversal in G, a rise followed by a fall, due to change of stimulus strength without change of frequency. Time in seconds.

allowing by either manipulation a preponderance of pressor influence. It is noticeable in this connection that on slow stimulation, even if strong enough to *decrease* the depressor effect of weaker stimulation (by counteraction due to higher threshold pressor fibers), the curve of depression is relatively horizontal (fig. 6, C) and only at higher frequencies is there a marked rise during stimulation after an initial fall. For the fall following

an initial rise on strong stimulation no obvious explanation occurs to us, unless the fact that the animal in the two cases where we have observed it went into gasping respiration that involved the whole body, accounts for the fall secondarily. It is perhaps significant that the vagus contains pain fibers activated in the same range of stimulus strength as the main group of pressor fibers (Heinbecker and O'Leary, 1933) while the depressor so far as we can observe has none.

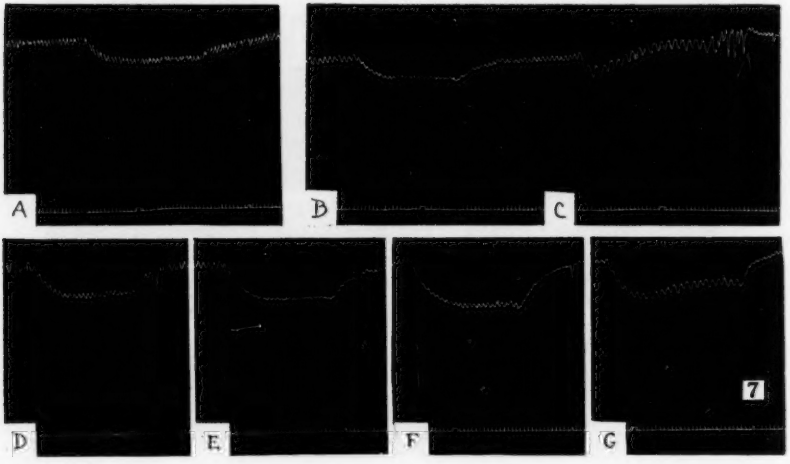

Fig. 7. A, Depressor, maximum induction coil stimulation 100/second. B, Vagus, induction coil, maximum at which pure depressor effect was obtainable. C, vagus, stronger stimulus than B. D-G, Vagus and depressor laid across same stimulating electrodes. D, 22 volts condenser charge, 16/second. E, 13.5 volts, 32/second. F, 13.5 volts, 48/second. G, 25 volts, 48/second. The relative strengths of stimulus for vagus alone in B and C and for vagus and depressor together in D–G can be judged from the effect on respiration; D, threshold effect. E, about as B. The depressor effect of A plus B equals E. G, respiratory effect slightly less than C. Depressor effect of A plus C equals G. The pure depressor effect of the depressor nerve sums with the vagus effects exactly as vagus pressor and depressor effects sum with each other.

Respiratory responses. On stimulation of the depressor, no immediate respiratory effect is obtained, but after 15 or more seconds there may be a gradual increase, presumably due to depletion of the center when the systemic pressure falls. In contrast to this the increase in respiration due to vagus stimulation is very prompt if the frequency is high. Often the threshold for respiratory increase is at the threshold for first action current from the nerve, but sometimes (figs. 4, A and 6, A and B) marked depression may be obtained via the vagus by high frequency stimulation, below

threshold for a significant respiratory effect. In general then we may conclude that respiratory accelerator fibers are distinct from vasomotor depressor fibers. We do not know whether they are distinct from pressor fibers, except that depressor nerves having an initial pressor action do not cause a corresponding increase in respiration when stimulated.

In practically all rabbits an increase of strength and frequency reverses an initial acceleration of respiration to a marked slowing. We have found it impossible to stop the respiration by any stimulation of one vagus in lightly anesthetized rabbits. It is very easy to stop the respiration if the depth of anesthesia is increased. With the degree of anesthesia which does not depress blood pressure, the results of increasing stimuli are first, increase of frequency and decrease of depth of respiration, then a reversal of these effects until the respiration may appear almost normal, except that less of a pause occurs between expiration and inspiration, and finally a greater slowing and deepening until gasping results. This is usually followed promptly by struggling movements, but whether this is due to potentially painful stimuli we have not ascertained; the latency of struggling is certainly much greater than that after similar stimulation of the saphenous for instance. While we have not yet investigated this reflex in detail, our results would permit of the inference that two groups of fibers are involved here also, with antagonistic functions, the accelerators lying in the larger fiber range, and the inhibitors in the small fiber range.

Discussion. With a central mechanism where temporal and spatial summation, that is, summation of successive impulses over the same fiber, and summation of coincidental impulses over parallel fibers, are equally effective, it may be said that the significant measure of the central effect is the total number of impulses arriving at the center, regardless of whether this number is determined by strength of stimulus or by number of repetitions (Bishop and Heinbecker, 1931, 1932; Gilson, 1931; Rosenblueth and Rioch, 1933). To these considerations should apparently be added the value of the impulse in a given fiber, which is a function of the fiber diameter, and the total time over which the impulses are distributed, which obviously must be short compared to the time over which the effect of a stimulus persists to make summation fully effective.

The summation of antagonistic impulses may be more complicated, but this may not be a complication that usually occurs in normal physiological function. When one set of end organs is stimulated normally, its antagonistic set is usually spared stimulation. The electrical stimulation of mixed nerves may therefore lead to an artifically confused picture of physiological functions, and we presume that the reversal of effect by simple increase of frequency is often such an abnormal complication. Unfortunately, in few nerves is it possible to find only one of the many pairs of reciprocal functions represented. Therefore the strength and frequency of stimulus become

of fundamental importance in interpretation of the results of artificial stimulation, and what might be termed the "functional content" of the nerve is an essential datum. Further experimentation is in order to determine how one group of impulses or another can be rendered ineffective in a mixed nerve, for adequate analysis of fiber function. Differential block or depression by anesthetics or by drugs offer possibilities if properly controlled. Reciprocal innervation may be the rule rather than the exception, in autonomic as well as in peripheral mechanisms.

The fact that the innervation involved in such a mechanism as that for vasomotor control is reciprocal, does not mean however that one effect is nothing more than the reversal of the other. From what we know of the relationships of the depressor nerve, for instance, it seems to be like the carotid sinus nerve mechanism, a device for internal regulation, an enteroceptive system, which is continuously in activity adjusting the systemic blood pressure. The pressor fibers of the saphenous, however, are more than the simple antagonists of the depressor nerve; they innervate the periphery of the body, are so located as to respond to external emergencies, and if their stimulation coincides under natural circumstances as it does under electrical stimulation with the stimulation of pain fibers, for which some evidence could be derived, their activity would involve adjustment to the more violent changes occurring in the environment. Under such circumstances the blood pressure might be raised in response to external conditions at the saphenous periphery, the rise of blood pressure stimulate the depressor endings internally, and the center then receive antagonistic impulses from both, quite as in electrical stimulation of both groups of fibers in such a nerve as the vagus. It is perhaps significant than when both types of fibers are stimulated strongly a rise of pressure predominates, that is, the requirements for the emergency overbalance the adjustments for a steady state. It is further possible that, while either pressor or depressor nerve fibers appear to cause a general change of systemic pressure, there may be some localization of effect, that is, the actual constricture or dilatation of vessels may occur in different regions of the body when different nerves are stimulated. This in fact occurs in the so-called Lovén reflexes (Lovén, 1866) where afferent stimulation may cause a general rise of systemic pressure, but a dilatation in the region from which the nerve arises.

SUMMARY

In responses to stimulations of the depressor nerve of the rabbit in preparations uncomplicated by the presence of pressor effects, the curves of action potential area against stimulus strength and of per cent depression of blood pressure against stimulus strength, recorded coincidentally, have a similar form.

The correction to be added to convert the curve of per cent lowering of blood pressure to a measure of afferent effect on the vasomotor centers is such as to suggest a direct proportionality between action curent area and central effect. This would mean that the effect per impulse varied as the action potential area per stimulus for fibers of different size. If area of potential varies as cross-sectional area of fibers, then the central effect per fiber varies as fiber mass.

The effect of stimulation of vagus and depressor vasomotor fibers together is equal to the sum of their separate responses, both as to amplitude and time course. The vasomotor afferent fibers of lowest threshold in the vagus are purely depressor. Of the vagus afferent vasomotors in the range of threshold of the depressor nerve fibers, the majority are pressor. When both are stimulated at slow frequency, the depression of blood pressure is less than when only depressors are stimulated at lower strength, but a horizontal curve of blood pressure may be maintained in both cases; if the frequency is increased, a fall is followed by a rise with the stronger stimulation, while a pure depression is obtained with the weaker.

The effect of frequency on stimulation of the vagus (or presumably of other mixed nerves) in giving a fall of pressure on slow stimulation, a rise on fast of the same strength, may be assigned to the stimulation of both pressor and depressor afferents in both cases, with different orders of temporal summation at the centers, such that the effect of increasing the frequency of pressor stimulation is greater than the effect on depressor stimulation.

There is some indication that respiratory afferents, acceleratory and inhibitory, resemble the vasomotors in their summation and antagonisms.

These statements apply to general systemic blood pressure effects, and the probability is recognized that this total may be made up of a combination of local reflexes whose analysis awaits further experiment.

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THE EFFECT OF INSULIN IN CONJUNCTION WITH POSTURE ON THE BLOOD CONCENTRATION IN DEPANCREATIZED DOGS[1]

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It has been suggested by the work of several investigators (1, 2, 3) that insulin when given in large doses and accompanied by a fall in blood sugar to a hypoglycemic level will cause blood concentration. This is of interest as anhydremia also occurs in diabetic coma, the treatment of which requires the administration of large amounts of insulin. In the work on dogs reported by Drabkin in 1924 (1) normal animals were used, they were anesthetized and the insulin was given intravenously. Hamilton, Barbour and Warner (4) could not corroborate Drabkin's original observations. As an index of blood concentration they used the specific gravity of the blood, whereas Drabkin had used the hemoglobin concentration. In 1925 Drabkin (3) repeated his work using unanesthetized normal dogs and measured the degree of anhydremia both by the hemoglobin and dry solid content of the blood. He confirmed his earlier observations that "after large doses of insulin, associated with a rapid, pronounced hypoglycemia, dogs invariably developed anhydremia." It would seem from these observations that the question of insulin anhydremia, at least, in dogs, had been settled. In the course of a study on the relationship of anhydremia to anuria in diabetic ketosis we were led to reinvestigate the question of the effect of insulin on the blood concentration in dogs. We found that when the animals were kept flat on the dog board and insulin was given, anhydremia occurred, but when the animals were allowed up during the experimental period, although the same degree of hypoglycemia followed the insulin administration, blood concentration did not occur. Drabkin makes no mention of the position of his animals during the experimental period, but we concluded that they were kept on the dog board, as in the first paper (1) they were anesthetized. Olmsted and Taylor (5) used decerebrated cats, so their animals were undoubtedly prone. In a study of fasting anhydremia Barbour and Frankman (6) state that "the simplest

[1] The insulin for this investigation was donated by the Eli Lilly Company.

way to obtain a smooth base line of blood concentration in dogs, is to keep the animals lying on one side, upon a comfortably padded table for at least one hour. Raising the head for an instant will definitely increase blood concentration slightly."

PROCEDURE. In this study we used depancreatized dogs except in experiments 11 and 12, table 3. The animals were kept on a standard diet of raw lean beef, cracker meal, glucose, a salt mixture and small amounts of dried yeast. As a measure of blood concentration hemoglobin determinations were done by the Newcomer method (8), hematocrit determinations were done in sedimentation tubes (9), and the dried total solids were estimated as previously described (7). All determinations were done in duplicate. The control determinations of total solids, hemoglobin and hematocrit are reported in actual values and the subsequent determinations are reported as per cent change from the control. In this way the magnitude of the change is more readily followed. From experiment 4 on, blood plasma sugars were done by the Folin method (10) on a Somogyi filtrate (11). Prior to experiment 4 the blood sugars were done by the Folin-Wu method (12) on whole blood. Blood was in all cases withdrawn from the external jugular vein. When the dogs were on the dog board during the experimental period, they were kept on their backs, the front and rear legs were held immobile. They were constantly attended, in order to keep them comfortable and quiet. The dog board was made of wooden slats and was not heated. No attempt was made to maintain a constant body temperature. The same dog board was used in all experiments, none of which were done during hot weather. During the experiments in which they remained upright, they were placed on the table only long enough to withdraw blood, a matter of about three minutes. They were then returned to their individual cages, and were watched to note any unusual position. In experiments 16 and 17 the animals were allowed up for the first two hours and kept flat for the last two hours. Insulin was given intramuscularly in doses large enough to produce hypoglycemia, usually this was seventy units. The dogs used weighed between 12 to 14 kgm. The dose was given in divided doses of 20 units, final dose 10 units, at intervals of twenty minutes.

RESULTS. In the first group of experiments, table 1, when the dogs were flat the results were similar to those obtained by Drabkin and his associates (12). The total solids rose in each experiment, except no. 4, where they fluctuated and where there was no hypoglycemia. In experiment 5 the blood sugar again remained high but the total solid concentration rose 9.1 per cent and remained up for three hours. We did not find the hemoglobin as reliable a guide of blood concentration, and we found this same fact true in studying the blood concentration in diabetic ketosis (7). Drabkin (2) reports rises in total solids, determined on blood from the

external jugular vein, of 9.2 per cent in 2½ hours with a fall of blood sugar from 88 mgm. to 0, and a rise of 14.8 per cent total solids in 2½ hours with a fall of blood sugar from 104 mgm. to 30 mgm. In experiments 1, 2 and 3

TABLE 1

Experiments with dog on dog board during experimental period. Insulin given

EXPT. NO.	1	2	3	4	5
DOG NO.	1	1	2	2	3
DIABETIC	Yes	Yes	Yes	Yes	Yes
INSULIN UNITS	50	75	70	70	70
1. Total solids per cent:					
Control	20.8	19.6	21.1	19.5	21.1
1 hour per cent change	+9.6	+12.2	+8.5	+11.8	+9.1
2 hours per cent change	+12.0	—	+11.8	+1.5	+5.2
3 hours per cent change	+8.4	+7.1	+9.9	+11.8	+5.2
5 hours per cent change	—	+6.6	+9.5	—	—
2. Hb.:					
Control per cent	95.0	88.0	81.7	90.0	90.0
1 hour per cent change	−1.1	+10.9	+6.1	−4.4	−8.9
2 hours per cent change	0.0	—	+22.4	−11.1	−15.5
3 hours per cent change	−3.0	+12.5	+22.4	−12.2	−21.1
5 hours per cent change	—	0.0	+19.8	—	—
3. Hematocrit cell volume:					
Control per cent	48.0	45.0	46.0	42.0	46.0
1 hour per cent change	0.0	+15.5	+6.5	+2.4	−2.2
2 hours per cent change	+10.4	—	+17.4	+2.4	−6.5
3 hours per cent change	+2.1	+6.6	+17.4	0.0	−17.4
5 hours per cent change	0.0	−2.2	+13.0	—	—
4. Blood sugar mgm:					
Control	169.	258.	393.	344.	318.
1 hour	117.	111.	307.5	415.	400.
2 hours	56.	63.	57.5	325.	350.
3 hours	44.	54.	42.	150.	213.
5 hours	65.	39.	28.	—	—

the total solids rose 9.6, 12.2 and 8.5 per cent in 2 hours and the blood sugars fell to 56, 63 and 57 mgm. respectively.

In the next group of experiments, table 2, insulin was given in the same doses but the animals were returned to their cages and remained standing or moving about in the cages during the experimental period. In none of

these experiments was there any rise in blood solids comparable to that which occurred in the first group of experiments. In spite of a definite

TABLE 2

Experiments with dog in cage during experimental period. Insulin given

EXPT. NO.	6	7	8	9	10
DOG NO.	3	4	5	5	4
DIABETIC	Yes	Yes	Yes	Yes	Yes
INSULIN UNITS	70	70	70	70	70
1. Total solids:					
Control per cent	18.8	20.7	18.8	18.5	18.1
1 hour per cent change	−3.7	+0.5	+3.7	+4.9	+0.6
2 hours per cent change	−5.3	0.0	+3.2	+5.4	+0.6
3 hours per cent change	−4.8	+0.9	+0.5	—	−2.2
5 hours per cent change	−7.4	—	−3.7	0.0	−7.2
2. Hb.:					
Control per cent	64	66	71	—	63
1 hour per cent change	+1.6	−1.5	−4.3	(66)†	−1.6
2 hours per cent change	+3.1	−3.0	−2.8	(66)	0.0
3 hours per cent change	0.0	—	−2.8	—	+1.6
5 hours per cent change	−6.2	0.0	−7.0	(65)	−7.9
3. Hematocrit cell volume:					
Control per cent	37	39	36	36	35
1 hour per cent change	−2.8	0.0	0.0	+2.8	+2.9
2 hours per cent change	0.0	+5.1	+11.0	+5.5	+5.7
3 hours per cent change	+2.8	+5.1	+8.3	—	+2.9
5 hours per cent change	−2.8	−18.0	+5.5	+8.3	−2.9
4. Blood sugar mgm.:					
Control	250.	400.	333.	387.	463.
1 hour	61.	378.	329.	198.	134.
2 hours	32.	324.	60.	72.	40.
3 hours	31.	318.	35.	71.	30.
5 hours	30.	323.	25.	330.*	20.

* Glucose given.

† Reported in per cent as no control reading was obtained.

hypoglycemia, there was a tendency for a fall in the total solids. In experiment 7, no hypoglycemia supervened.

In the third group of experiments, table 3, insulin was not given and the dogs, three of whom were depancreatized and two normal, were kept

on the dog board for the entire experimental period. In the two normal dogs, experiments 11 and 12, there was no evidence of blood concentration and the blood sugars remained practically constant. In the depancrea-

TABLE 3

Experiments with dog on dog board during experimental period. No insulin

EXPT. NO.	11	12	13	14	15
DOG NO.	6	7	2	2	7
DIABETIC	No	No	Yes	Yes	Yes
INSULIN	No	No	No	No	No
1. Total solids:					
Control per cent	—	21.	21.1	21.9	21.4
1 hour per cent change	(19.7)*	−3.3	+5.7	−2.3	−3.7
2 hours per cent change	(19.7)	−6.7	−1.5	+2.2	−3.3
3 hours per cent change	(18.7)	−8.6	—	—	−3.7
5 hours per cent change	(19.0)	−8.1	—	—	—
2. Hb.:					
Control per cent	86	90	86	100	86
1 hour per cent change	−11.6	−8.9	−2.3	−10.0	−4.7
2 hours per cent change	−8.1	−15.5	−4.7	−5.0	−3.5
3 hours per cent change	−8.1	−21.1	−4.7	−14.0	0.0
5 hours per cent change	−8.1	−15.5	0.0	−20.0	—
3. Hematocrit cell volume:					
Control per cent	44	46	44	46	57
1 hour per cent change	−9.1	−2.2	+9.0	−4.4	−19.3
2 hours per cent change	−6.9	−8.8	0.0	+4.3	−22.8
3 hours per cent change	−6.9	−17.3	0.0	+6.5	−17.5
5 hours per cent change	−13.7	−13.0	+2.2	−6.5	—
4. Blood sugar mgm.:					
Control	100.	92.	278.	225.	549.
1 hour	107.	103.	333.	324.	621.
2 hours	101.	102.	378.	366.	599.
3 hours	106.	97.	432.	378.	567.
5 hours	109.	98.	438.	402.	—

* Reported in per cent as no control reading was obtained.

tized dogs there was a slight rise in the total solids in experiments 13 and 14, which was not sustained and in experiment 15 there was a fall. Naturally the blood sugars remained high, which probably influenced the state of blood hydration. If these experiments are compared to those in which

hyperglycemia persisted, despite the administration of insulin, no. 5 when the dog was flat and no. 7 when the dog was upright, it will be noticed that the total solid content in the former rose 9.1 per cent and remained

TABLE 4

Experiments with change of position during experimental period. No insulin

Dog upright for 2 hours. Then flat in experiments 16 and 17: On dog board for 1 hour and upright for 1 hour in experiment 18.

EXPT. NO.	16	17	18
DOG NO.	2	2	2
DIABETIC	Yes	Yes	Yes
1. Total solids:			
Control per cent	21.4	21.4	21.1
1 hour per cent change	−2.9	−3.3	0.0
2 hours per cent change	−1.4	−4.7	−5.1
3 hours per cent change	+18.2	−1.0	—
5 hours per cent change	+4.0	0.0	—
2. Hb.:			
Control per cent	97	92	84
1 hour per cent change	−8.3	−6.5	0.0
2 hours per cent change	−11.3	−10.9	−14.3
3 hours per cent change	−7.3	−2.1	—
5 hours per cent change	−7.3	−4.3	—
3. Hematocrit cell volume:			
Control per cent	44		41
1 hour per cent change	−4.5	(39.5)*	+9.7
2 hours per cent change	−2.3	(39.0)	0.0
3 hours per cent change	+6.8	(42.0)	—
5 hours per cent change	+6.8	(42.0)	—
4. Blood sugar mgm.:			
Control	577.	364.	
1 hour	521.	396.	
2 hours	515.	387.	
3 hours	528.	411.	
5 hours	540.	369.	

* Reported in per cent as no control reading was obtained.

for three hours higher than the control, whereas in experiment 7 the total solid remained practically unchanged for three hours.

In the fourth group of experiments, table 4, the depancreatized dog was

used. In experiments 16 and 17 the dog was upright for the first 2 hours and then was on the dog board for two hours. No insulin was given. One hour after being flat the total solids, hemoglobin and cell volume all rose. This rise was maintained for the next two hours. A hyperglycemia was always present. In experiment 18 the dog was flat for the control and the one hour period, and then returned to her cage. The total solids, hemoglobin and cell volume all fell slightly.

Discussion. From these observations it would seem that the blood concentration which accompanies hypoglycemic doses of insulin depends, in the dog, on the position of the animal. We did not use doses of the same magnitude as Drabkin, but the doses used cause hypoglycemia, which when the animals were in a position, normal for the species, was not accompanied by anhydremia. Another possible factor to be considered is skin evaporation. We do not, however, think this explains the anhydremia when the dog was on its back and received insulin as it seems reasonable that this would have obtained equally well when the dog was on its back and *did not* receive insulin. Unless one wishes to argue that insulin increases skin evaporation, in which case it should have operated as a factor when the dog received insulin and was upright as the temperature of the room was the same in both instances. Drabkin (1, 2) incidentally does not discuss this possibility, nor is there any indication in the report of his experiments of any effort to control body temperature. Keith (13) in a study of experimental dehydration found that from 7 to 10 per cent of the animal's entire store of water could be excreted without any significant change in body temperature. With this degree of dehydration there was definite blood concentration. We followed the respiratory rate per minute in the experiments with and without insulin when the dog was on the dog board. In the experiments with insulin the respiratory rate rose 44 per minute. When no insulin was given there was no change in one experiment and in the other experiments the average of the greatest increases was 21 per minute. This shows that with insulin when the dog was on its back there was a loss of water and is added evidence that the blood concentration is not due to skin evaporation. It was interesting to us that position alone caused no marked change in blood concentration. This is understandable in the depancreatized dog with an elevated blood sugar, but is more difficult to explain in the normal dog. Barbour et al. (6) were apparently aware of some effect of change of posture as they state very carefully their criteria for establishing a base line for blood concentration. Also in our last group of experiments, table 4, when the position was changed during the experimental period, changes in blood concentration were observed. It is difficult to explain these fluctuations, which were only observed in the diabetic animal.

Apparently, however, insulin has some ability to interfere with the

exchange of fluid between the tissues and the blood, in the depancreatized animal when kept on its back.

SUMMARY

1. In three experiments hypoglycemic doses of insulin were accompanied by blood concentration when the dogs were kept on their backs during the experiments.

2. In four experiments with the animal upright hypoglycemic doses of insulin were not accompanied by blood concentration.

3. Keeping the animals flat without administering insulin did not cause blood concentration in five experiments.

4. In three experiments, where the position was changed during the experimental period, slight changes in blood concentration occurred.

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NUTRITIONAL SIGNIFICANCE OF VITAMIN A THROUGHOUT THE LIFE CYCLE

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Evans (1922, 1928) has observed that inadequate vitamin A in the food of female rats resulted in a characteristic disturbance of reproductive function, while experiments by Sherman and MacLeod (1925) have shown that, when parallel groups of rats of identical previous history were fed on diets one rather low and one fairly high in vitamin A, both seemed sufficient for normal growth but the poorer diet was not adequate for reproduction and the rearing of young nor for satisfactory longevity. Sure (1930) has reported that in five generations of rats the average percentage of young successfully weaned increased in proportion to increases in the butterfat content of his diets in the range from 5 to 11 per cent butterfat.

The experiments described here were designed to ascertain whether differences in nutritional wellbeing would appear when rats were fed diets containing amounts of vitamin A intermediate between those employed by Sherman and MacLeod. The diets used were in effect modifications of diet B ($\frac{2}{3}$ whole wheat and $\frac{1}{3}$ whole milk powder, salt, 2 per cent of the wheat) which was first described by Sherman and Muhlfeld (1922) and has been continuously studied since, by Sherman and co-workers. In the modified diets whole milk powder was replaced by skimmed milk powder plus butterfat (or butterfat and Crisco mixtures). The diets contained 8, 4, 2, 1, $\frac{1}{2}$ and 0 per cent butterfat, the total fat content being kept at 8 per cent by the inclusion of Crisco. Extra vitamin D was furnished (except in the earliest experiments on 8 per cent and 4 per cent butterfat) by irradiating the rats with ultra violet light.

Each decrease in butterfat in the original diets resulted in a distinct decrease in survival (indicating decrease in stored vitamin A) when rats of the second generation were placed on a vitamin A-free diet, the survival periods being 56, 44, 32, 24, and 19 days for 8 per cent, 4 per cent, 2 per cent, 1 per cent, and $\frac{1}{2}$ per cent butterfat, respectively. All these differences were more than 5 times their probable errors (Chaddock, 1925) and may reasonably be considered as significant. It is interesting to observe that in each case longer life was accompanied by greater gain before maximum weight was attained. There is, therefore, unmistakable evidence of

a difference in bodily store of vitamin A even between the two diets highest in butterfat content, the larger amount of butterfat resulting in a larger bodily store both at one month and at three months of age.

Each decrease in butterfat below 4 per cent resulted in slower growth and in lower adult weight at all ages (fig. 1). In reporting results the data have been divided into groups so that, in all cases, only litter mates of the same sex have been directly compared. Above 4 per cent there was no consistent difference in the growth rate or in the maximum weight attained by the experimental animals. The difference between the animals on 4 per cent and 2 per cent butterfat diets was very slight.

Table 1 gives the results of the breeding and length of life records discussed hereafter.

The average age at death declined as the vitamin A content of the diet was lowered, the decrease found between 1 per cent and ½ per cent butterfat being 6 to 7 times its probable error. In view of all collateral evidence the other differences, though too small to be statistically conclusive, are probably significant in fact.

A significant difference in the weight of young at 28 days was found between rats on diets containing 4 per cent and 2 per cent butterfat, in the number of young reared below 2 per cent, and of young born below 1 per cent butterfat. Rate of growth, duration of reproductive life, and length of life also showed a statistically significant decrease in rats on diets containing less than 1 per cent butterfat.

Fourth generation rats were successfully reared on the diets containing 8 per cent, 4 per cent, and 2 per cent butterfat, at which time the reproduction experiments were terminated. Fourth generation rats were born, but only a small proportion reared, on 1 per cent butterfat, and a very few third generation rats were born but not reared on ½ per cent butterfat.

The differences mentioned above are only those which may be considered statistically significant. The numerical value of every comparison shows a decrease with every decrease in butterfat below 4 per cent and the ratios of the differences to their probable errors are seldom less than one, so that it is possible that further experiment might prove differences to be significant which may here be considered merely suggestive.

Autopsies show that pus-like material at the base of the tongue appeared in 95 per cent of the animals which had received a vitamin A-free diet from the age of 28 days until death. Similar material appeared in the lungs but not in the tongues of rats which received larger amounts of vitamin A. About 65 per cent of the rats on diets containing 1 per cent and ½ per cent butterfat and about 90 per cent of those on diets containing 8 per cent, 4 per cent, and 2 per cent butterfat showed this pus-like material in the lungs. In other words, evidence of an adverse effect upon the lungs of rats on diets low in vitamin A appeared only when survival was longer than that

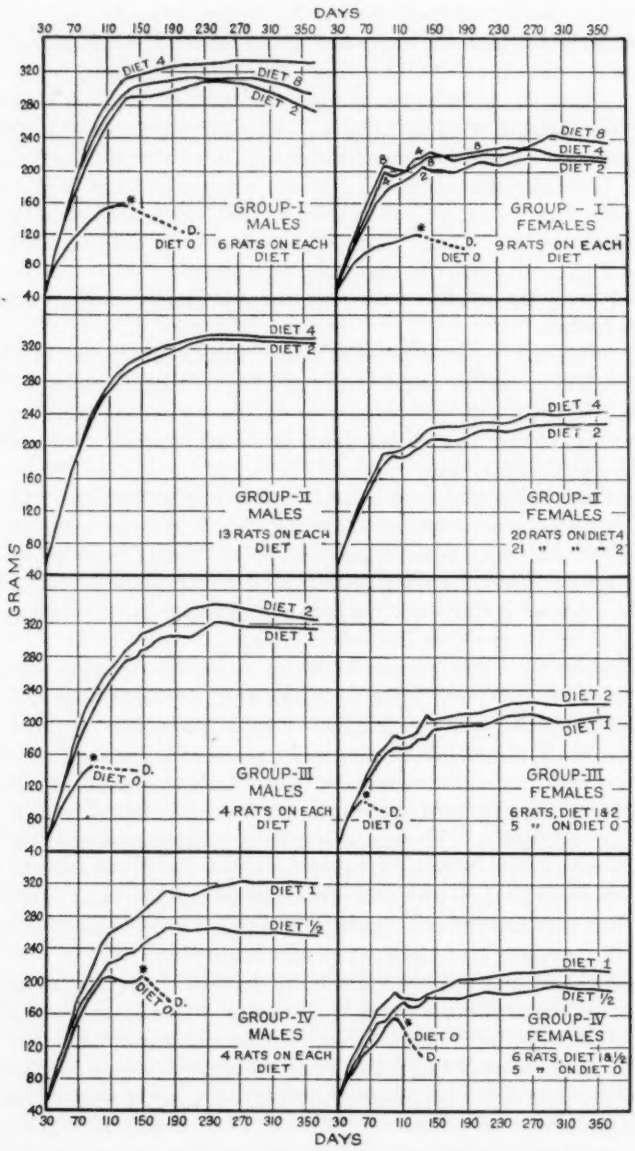

Fig. 1. Average weights, up to one year of age, of rats kept on diets of different butterfat content. The beginning of a broken line indicates that one or more rats have died. The broken line terminates at the point representing the average weight and age at death, *D*.

The diet numbers correspond to the per cent of butterfat in each diet.

of rats subjected to a drastic deficiency of vitamin A. The immediate cause of death was not definitely established in any case, but the general breakdown resulting in death, though accompanied by similar symptoms at all higher levels of vitamin A, was postponed to successively later ages as the

TABLE 1

Comparison of rats fed diets containing different amounts of vitamin A

DIET NUMBER	NUMBER OF RATS		REPRODUCTION RECORD, AVERAGE PER FEMALE				FEMALES WHICH BORE YOUNG	LENGTH OF LIFE			STORAGE OF VITAMIN A IN 2ND GENERATION	
	Male	Female	Young born	Young reared	Weight young 28 days	Reproductive period		Male	Female	All	Number	Survival on vitamin A-free diet
					grams	*days*	*per cent*					
8	6	9	22.8	7.0	51.2	160	89	570	711	654	19	55.9
4	6	9	15.7	10.2	51.3	119	67	596	628	615	35	43.8
Difference.....			7.1	−3.2	−0.1	41		−26	83	39		12.1 ±2.0
4	13	20	21.8	15.0	49.5	186	75	629	715	681	35	43.8
2	13	21	18.1	13.4	47.5	147	76	602	648	630	29	32.3
Difference.....			3.7 ±3.7*	1.6 ±2.8	2.0 ±0.5	39 ±29		27	67	51 ±28		11.5 ±1.5
2	4	6	30.5	22.0	43.5	271	100	602	844	747	29	32.3
1	4	6	25.5	10.2	41.0	242	100	570	792	703	20	24.1
Difference.....			5.0 ±4.4	11.8 ±3.5	2.5 ±0.8	29 ±34		32	52	44 ±65		8.2 ±1.1
1	4	6	28.8	9.8	42.8	236	100	702	638	664	20	24.1
½	4	6	11.2	1.8	41.3	85	100	462	449	454	11	19.0
Difference.....			17.6 ±2.9	8.0 ±2.9	1.5 ±1.5	151 ±22		240	189	210 ±41		5.1 ±0.6
½	4	6	11.2	1.8	41.3	85	100	462	449	454		
0	4	5	0.8	0.0		8	40	163	156	159		
Difference.....			10.4 ±1.2			76 ±14		299	293	295 ±23		
1	6	9	28.2			232	100			683		
0	6	8	0.5			5	25			119		
Difference.....			27.7 ±2.0			227 ±14				564 ±27		

* Probable errors calculated according to Chaddock, 1925.

vitamin A content of the diet was increased. Results reported by Mendel and Vickery (1928–1929) lend support to this conclusion. Urinary calculi were visible in rats on diets very low in vitamin A but not in rats receiving no vitamin A at all. This result is in accord with the findings of VanLeersum (1928) and of early observations of Osborne and Mendel (1917) who

report, respectively, microscopic deposits in the kidneys of rats on a vitamin A-free diet, and macroscopic renal calculi in rats on diets low in but not completely devoid of vitamin A. This is doubtless because on diets entirely devoid of vitamin A the animals die before there is time for the calculi to become large.

From the point of view of general nutrition, 8 per cent butterfat apparently supplies more than sufficient vitamin A for the requirements of growth and successful reproduction without inducing an inconveniently large store in animals to be used for vitamin A tests.

Our assumption that the relative vitamin A values of these diets would correspond to their relative butterfat content was supported by the results of tests on the food materials used during the experiment. When whole wheat replaced all the starch (67 per cent) in the vitamin A-free diet, standard vitamin A test animals (Sherman and Batchelder, 1931) lived only 5 days longer than their negative controls. When 60 per cent skimmed milk powder was fed in the same way, the test animals lived only ten days longer than their negative controls. Rats receiving 60 per cent of their calories from Crisco lived no longer than their negative controls. On the other hand, rats receiving $\frac{1}{2}$ per cent butterfat (fed separately from the basal diet) showed a net gain of 39 grams in 8 weeks so that it is apparent that whole wheat, skimmed milk powder, and Crisco in the experimental diets contributed negligible amounts of vitamin A as compared with even the smallest percentage of butterfat used. In fact, the known response of standard rats to graded doses of vitamin A (Sherman and Batchelder, 1931) indicates that the total amount of vitamin A contributed by all other ingredients of the diets used in the present experiment was less than one-eighth the amount contributed by the lowest percentage ($\frac{1}{2}$ per cent) of butterfat used. The same brand of tub butter, purchased at retail as needed, throughout several years, showed comparatively small variations in vitamin A content at different times and seasons.

These results indicate that the vitality of rats is affected in various ways by a deficiency of vitamin A, each successive diminution of vitamin A in the diet resulting in increasing evidence of nutritive failure. This study of animals on widely differing amounts of vitamin A in an otherwise adequate (though not optimal) diet emphasizes the fact that our judgment of the adequacy of any diet should be based on as many measures of nutritional success as possible. Only in this way can we be sure that our estimate of necessary amounts meets all needs of the body including growth, repair, reproduction, lactation, and length of life.

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"THE FAT METABOLISM HORMONE" AND HYPERGLYCEMIA

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In preliminary publications (1) we have pointed out that a hyperglycemia and an increase of acetone bodies in the blood can be produced by the injection of an extract obtained from male urine. We now wish to report a more extensive series of investigations, which also include observations on carbon-dioxide combining power and lactic acid in the blood.

First, as to the method of preparation of the extract, still admittedly very crude. We use, essentially, the method adopted by Funk (2) for the preparation of an extract which when injected into rats gives rise to an increase in the acetone bodies in the urine (Funk's "fat-metabolism" hormone), and which method is practically identical with that used by Katzman and Doisy (3) for preparing an extract which, when injected into rabbits, gives rise to a hyperglycemia. Because of modifications which have been introduced, we shall give the exact details of the method: Using 3 liters of urine, freshly collected and preserved with thymol, it is first made acid to methyl red with glacial acetic acid. Ninety grams of benzoic acid are dissolved in 180 cc. of 95 per cent alcohol, with the aid of heat, and the solution of benzoic acid is added, slowly, and very little at any one time, to the urine, which is continuously stirred. The precipitate is allowed to settle for about 15 minutes and then rapidly filtered with the aid of suction. The well-pressed precipitate is suspended in 180 cc. of 95 per cent alcohol, stirred thoroughly and the whole then centrifuged for 15 minutes. The supernatant liquid (containing mainly benzoic acid, which is recovered) is poured off, 90 cc. of 95 per cent alcohol is added to the residue, stirred and again centrifuged for 15 minutes. The supernatant liquid is poured off, the residue is transferred to a small beaker with the help of 3 to 5 cc. of *N* NH_4OH, stirred and tested if alkaline to phenolphthalein. If it is not, concentrated NH_4OH, one drop at a time, is added, until the suspension reacts alkaline. The suspension is centrifuged for 15 minutes. The solution is again tested with phenolphthalein. If the alkalinity has disappeared, as sometimes happens, the solution is made alkaline with concentrated NH_4OH and again centrifuged. The final product should be alkaline to phenolphthalein. The supernatant liquid (which is usually very far from clear) is poured off and made up to 10 cc.

with distilled water. Before injection, enough acetic acid is added to make the solution just acid to phenolphthalein.

TABLE 1

Effect of the injection into rabbits of the urine preparation

RABBIT NUMBER	AMOUNT INJECTED (EQUIVALENT IN LITERS OF URINE)	TIME (IN HOURS) OF MAXIMUM CHANGE AFTER INJECTION	BLOOD GLUCOSE, NORMAL, MGM. PER 100 CC.	BLOOD GLUCOSE, MAXIMUM, MGM. PER 100 CC.	BLOOD GLUCOSE, PER CENT INCREASE	TOTAL ACETONE BODIES, NORMAL, MGM. PER 100 CC.	TOTAL ACETONE BODIES, MAXIMUM, MGM. PER 100 CC.	TOTAL ACETONE BODIES, PER CENT INCREASE	CO_2 COMBINING POWER, NORMAL, VOLS. PER CENT	CO_2 COMBINING POWER, MINIMUM, VOLS. PER CENT	CO_2 COMBINING POWER, PER CENT DECREASE	BLOOD LACTIC ACID, NORMAL, MGM. PER 100 CC.	BLOOD LACTIC ACID, MAXIMUM, MGM. PER 100 CC.	BLOOD LACTIC ACID, PER CENT INCREASE
1	3.0	3.5	126	353	180									
2	2.0	6	94	144	53									
3	1.8	3	144	180	25	35	135	286						
4	3.0	3	98	155	58	10	17	70						
5	5.0	2	84	140	67									
6	5.0	3	105	322	207									
7	2.4	0.5	112	133	19	32	61	90						
8	3.0	1.5	90	165	83	19	74	289						
9	2.6	1.5	103	152	48	44	76	73						
10	2.9	3	92	170	85	14	32	129						
11	2.6	3	95	185	95	20	39	95						
12	3.0	2	94	286	204	50	111	122						
13	3.0	3	110	357	325									
14	4.3	3	123	242	96	95	233	145						
15	4.3	2	141	193	36									
16	3.0	1	106	111	4									
17	3.0	3	106	122	15				22	18	18			
18	3.0	3	111	240	116				22	9	69			
19	2.7	3	126	131	3									
20	3.0	3	112	118	5									
21	2.3	3	112	265	136				30	13	57	5	16	215
22	3.0	3	111	156	40				26	13	50	8	12	48
23	3.6	3	120	197	64				28	15	46			
24	3.6	2	146	230	57				22	10	55	5	12	127
25	3.2	0.5	97	105	8				30	20	34	7	8	14
26	3.2	4	120	170	42				32	14	56	6	8	34
27	5.5	2	114	279	118				27	6	78	5	12	140
28	6.0	3	106	230	117				22	4	82	6	12	100
29	5.3	4	115	205	78				19	7	63	6	16	167

Ten cubic centimeters, then, of the solution represent 3 liters of urine.

The results are given in the appended table. At first we confined ourselves to blood sugar and blood acetone; later, mainly because of the

difficulties in determining blood acetone, we substituted CO_2-combining power, and also added lactic acid to the list of determinations.

It will be noticed that, with very few exceptions, large increases in blood sugar, acetone bodies and lactic acid, and notable decreases in CO_2-combining power were obtained. Whatever the substance may be (hormone or otherwise) its effect on carbohydrate and fat metabolism is profound. It may be remarked that where negligible percentage changes are recorded, the results were invariably due to allowing the urine to stand too long before use, or to delay in the completion of the preparation.

Controls (using 10 cc. of physiological saline solution, to which enough NH_4OH was added to make it alkaline to litmus) were also injected. Here are the results.

	BLOOD SUGARS	CO_2 COMBINING POWER	BLOOD LACTIC ACID
		vols. per cent	*mgm. per 100 cc.*
Before injection	114	30	6.3
½ hour after	115	32	6.4
1 hour after	114	31	6.3
2 hours after	114	30	6.5
3 hours after	113	29	6.6
4 hours after	113	28	

So far, all attempts to show increased excretions of glucose and acetone bodies in the urine have failed. We first used rabbits and later rats. Twenty-four hour samples were collected and analysed.

The methods used for determinations were as follows: for blood sugar, the Folin and Wu (4); total acetone bodies in blood, Van Slyke[1] (5); Co_2 combining power, Van Slyke (6); and lactic acid in blood, Avery and Hastings (7); for glucose in the urine, Shaffer-Hartman, as modified by Somogyi (8), and the acetone bodies in the urine, Van Slyke (9).

CONCLUSIONS

1. A preparation can be obtained from urine (male) which, when injected into rabbits, causes a hyperglycemia, an increase of acetone bodies and lactic acid in the blood and a decrease of CO_2-combining power.

2. Apparently there is a disturbance of carbohydrate and fat metabolism.

[1] For practical reasons, 1 cc. instead of 10 cc. of blood was used in these acetone determinations. The accuracy of the method, using 1 cc., is probably not above reproach; this led us later to substitute CO_2-combining power.

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STUDIES ON THE NERVOUS SYSTEM IN DEFICIENCY DISEASES: EXPERIMENTAL BLACK TONGUE[1]

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In spite of much research on the problem, human pellagra continues to take a large toll of deaths annually and the exact etiology of the disease remains a subject of debate. As a result of their studies, Goldberger and his associates (1) suggested that the canine disease "black tongue" is the analogue of pellagra in man, and that this condition is essentially a dietary deficiency, probably a lack of vitamin B_2(G). "Black tongue" is characterized by cutaneous, lingual and buccal lesions and by diarrhea and salivation.

The diets used by Goldberger and other workers on the problem are faulty in several respects, and therefore Cowgill, Zimmerman and Burack (2) attempted to reproduce the canine condition with an artificial dietary regimen in which individual constituents were more carefully controlled and which was deficient, as far as is known at the present time, only in vitamin B_2(G). The results of that study were surprising in that, contrary to expectation, a condition quite dissimilar to black tongue was produced. The pathologic changes in these dogs were reported by Zimmerman and Burack (3). Oral lesions characteristic of so-called black tongue were lacking in all these animals; only one had slight ulcers on the buccal mucous membranes, and one other exhibited the characteristic pronounced salivation. In contrast to the customary rapid onset and progression of black tongue, the disease-complex produced by the artificial ration developed slowly. The anatomic changes referable to the nervous system consisted of marked demyelination of the peripheral nerves, the posterior and anterior spinal nerves, and the posterior columns of the spinal cord. Attention was called to the fact that these degenerative changes in the nervous system were similar to or identical with those frequently described in human pellagra.

[1] A part of the expense of this investigation was defrayed by a grant from the Research Fund at Yale University School of Medicine.

[2] Sterling Fellow in Physiological Chemistry, 1932–1933.

Goldberger and his colleagues, on the basis of the similarity of the clinical pictures of black tongue in dogs and pellagra in man, entertained the view that these conditions were analogous. We have then at least two conditions in dogs, namely, black tongue and the disease-complex described above when the animals subsist on an artificial ration lacking only vitamin B_2(G), which are themselves dissimilar but which stand each in a definite relationship to human pellagra. To find any further associations between these two canine conditions, it was at once apparent that the nervous systems of dogs with black tongue must be examined with the same methods employed in the vitamin B_2(G) experiments previously referred to (3). The present communication presents in part the results of such a study.

TABLE 1

		PROTEIN	FAT	CARBOHYDRATE
	gm.	*gm.*	*gm.*	*gm.*
Corn, whole white, ground*	400	33.6	18.8	296
Cow peas, ground*	50	10.7	0.7	30.4
Casein, purified†	60	52		
Sucrose	32			32
Cottonseed oil	30		30	
Cod liver oil	15		15	
Salt mixture‡	22			
Total nutrients, gm	609	96.3	64.5	358.4
Total calories	2465.1	395.8	599.9	1469.4

* Corn and peas were purchased as whole grains from a local dealer and were ground in the laboratory. The desired quantity of these was cooked in water for 1½ hour before it was mixed with the other ingredients. Enough water was added to the whole mixture to make the total weight equal to 2400 grams, so that 1 gram of the moist food was equivalent to about 1 calorie.

† Casein was purified by repeated leaching with dilute acetic acid, as recommended by McCollum et al. (5).

‡ Osborne and Mendel (6).

This research was also undertaken to supplement the observations of Cowgill and Gilman (4) which appeared to indicate that no derangement of gastric function accompanies subsistence on the Goldberger diet number 195 and thus failed to substantiate the idea that the deficiency involved in this ration has any relation to the syndrome of combined system disease. Finally, observations of hematopoietic changes made in these animals will be summarized briefly.

EXPERIMENTAL PROCEDURE. Six mongrel, adult dogs were fed diet number 195 of Goldberger, Wheeler, Lillie and Rogers (1) for periods which varied from 64 to 155 days. The food mixture consisted of the ingredients listed in table 1.

Two control animals were maintained for 154 and 192 days respectively on the same diet, with the daily addition of 8 grams of dried yeast.[3] Food was supplied ad libitum, and the amount voluntarily ingested by each animal was recorded daily. The dogs were housed in large wire cages in the same animal house and under environmental conditions identical with those of the animals used in the vitamin B_2(G) study already reported (2, 3). All of the dogs were examined for gross manifestations of disorder and weighed about three times each week. At frequent intervals gastric juice was removed and analyzed for pepsin and for free and combined hydrochloric acid. Examinations of the blood, including the total number of red and white blood cells, the amount of hemoglobin and the differential count of white blood cells, were made on three of the dogs every three or four days. The typical "black tongue" condition, agreeing with the

TABLE 2

DOG	SEX	ORIGINAL WEIGHT	LOSS OF WEIGHT	PER CENT LOSS IN WEIGHT	APPETITE	TOTAL DAYS FROM START OF DIET TO OCCURRENCE OF:				REMARKS
						Anorexia	Diarrhea	Oral lesions	Death	
		kilos	*kilos*							
69	M.	14.3	8.1	57	Erratic	54	56	65	155	Signs disappeared and recurred twice
70	M.	11.6	6.4	55	Erratic		72	122	123	
74	F.	6.7	2.2	33	Excellent	60	59	61	64	
142	M.	11.7	4.0	34	Excellent	78	44	67	83	One remission and recurrence
155	F.	9.3	5.6	60	Good	48	45	84	119	
157	F.	7.3	2.8	37	Good	102		116	122	Vomiting and salivation on 61st day

description given by Goldberger and associates, was readily produced in the experimental animals, but did not appear in the control dogs.

Complete necropsies were performed on all the animals, and the viscera were examined both in the gross and with the aid of microscopic preparations. The central and peripheral nervous systems were subjected to the same careful examination employed in the experiments of Zimmerman and Burack (3). Microscopic preparations of these tissues were stained with toluidine blue and with Sudan III and by the Spielmeyer, Bielschowsky, Marchi and Kulschitzky methods.

RESULTS. In spite of care to supply a liberal amount of fresh food daily, individual variation in food consumption of the experimental dogs was large. So far as could be determined, there was not a definitely quantitative relation between the food intake and the varying loss in body

[3] Northwestern Yeast Company, Chicago, Illinois.

weight which occurred. The control animals maintained excellent appetites and practically constant body weight for the entire duration of the experiment. The time relations of the appearance of the various signs in each dog, as well as the losses in weight, are indicated in table 2.

Necropsies. Of the three animals which died during the course of the experiment, dog 70 revealed pulmonary abscesses at necropsy, dog 142 died of intussusception and gangrene of the terminal portion of the ileum, and dog 157 had an organizing pneumonia which was apparently the result of aspiration of foreign material. In all six animals the gastro-intestinal tract was the seat of numerous ulcerative and hemorrhagic lesions, and four of the six dogs had ulcers and hemorrhages in the gingival and buccal mucosae. The walls of all the ulcerative lesions were infiltrated with

Fig. 1

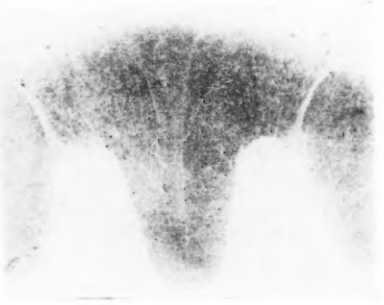

Fig. 2

Fig. 1. Posterior columns of spinal cord of dog 157. Note small number of degenerated medullary sheaths stained black. Marchi method; ×15.

Fig. 2. Posterior columns of spinal cord of dog 198 (control animal). Marchi method; ×15.

polymorphonuclear leucocytes and lymphocytes. On the periphery of some of the gastric ulcers were found thrombosed arterioles.

In the gross, neither the central nor the peripheral nervous systems of any of the animals showed pathologic changes. On histologic examination the peripheral nerves of dogs 70, 74 and 142 failed to reveal degenerative changes by any of the staining methods employed, whereas those of dogs 69, 155 and 157 showed a slight amount of demyelination by the Marchi method. A small number of medullary sheaths in the posterior columns (Goll and Burdach) were destroyed at all levels of the spinal cords of dogs 70, 155 and 157 (fig. 1). In the Nissl preparations of the spinal cord of this last animal, a proliferative reaction of the glia was found in the posterior columns, which appeared to be replacing the destroyed medullary sheaths. None of the other staining methods revealed any pathologic alterations

in these or other parts of the nervous system. It is to be noted that in general even this slight degree of degeneration in the nervous systems occurred only in those animals which survived the longest. Equally careful examinations performed on the two animals used as controls (dogs 197 and 198) did not reveal any lesions in the mucous membranes, the gastrointestinal tracts or the nervous systems (fig. 2).

Analysis of gastric juice. The response of the gastric secretion to histamine was tested in five of the experimental dogs and in the two controls. In dogs 69, 70 and 74 tests were made both before and during the condition of black tongue. Because of the method of obtaining the juice, by stomach sound, no attempt was made to judge the volume of the flow except to note that in every case enough juice could be obtained without difficulty for the analysis of free and combined hydrochloric acid and of pepsin. These analyses were kindly made for us by Dr. E. R. B. Smith, who found that in every instance except one (dog 70 on the day of its death) there was actually more free acid present than in the average of the two controls, while pepsin values were within the normal range. There was no significant decline in these constituents of the gastric juice during the progress of the disease in any animal except dog 70, which showed a loss of free acid.

Examinations of blood. Blood was removed from the ear veins of dogs 69, 70 and 74 and was examined once or twice weekly. During the early part of the experiments, before the onset of diarrhea, the red blood cells in each animal numbered between 4.8 and 7.1 millions per cubic millimeter, the white blood cells ranged between 5.5 and 13.8 thousands per cubic millimeter, the hemoglobin between 14.5 and 16.0 grams per 100 cc. of blood (Hayden-Hauser), and the reticulocytes from less than 1 to 4 per cent; erythroblasts were seen but rarely, and the differential count of white blood cells was not unusual in any way. With the advent of diarrhea and the progression of the disease the total number of red blood cells fell slightly; they began to appear achromic and the hemoglobin fell to about 10 grams per 100 cc. of blood. There was no change in size of the red blood cells. The color index remained between 0.8 and 0.9. There was no appreciable increase in the numbers of reticulated red cells and erythroblasts. On several occasions the number of white blood cells rose to 20,000 per cubic millimeter, at which time there was a corresponding rise in the number of polymorphonuclear leucocytes. The leucocytosis was associated with evidence of inflammatory lesions in the buccal mucous membranes.

An interesting observation was made of the effect of ferric and ammonium citrate (U. S. P.) on the blood picture of dog 69. This animal had a steady drop of the red blood cells from over 7 million to 4.6 million per cubic millimeter and a corresponding fall in hemoglobin from 15 to 10 grams, during a period of 110 days. The daily administration of 1 gram of the ferric salt produced a prompt but slight rise in both the number of

erythrocytes and hemoglobin. When the amount of ferric and ammonium citrate administered was raised to 10 grams per day, there was a return to practically normal values of both factors. It is, however, worthy of note that the disappearance and recurrence of the signs of black tongue in this dog bore no relation to the time at which iron therapy was instituted.

Discussion. Black tongue in the dog admittedly has many striking similarities in its clinical features with the condition known as pellagra in man. Hemorrhagic and ulcerative lesions in the gastro-intestinal tract are also found in both conditions with great frequency. Another frequent manifestation of pellagra, however, consists of degenerative changes in the posterior, lateral and anterior columns of the spinal cord. They are the changes which were reproduced so closely in the dog by Zimmerman and Burack (3) with diets lacking the water-soluble, heat-stable vitamin B_2(G), changes which are almost completely absent in the animals of the present study. The slight degree of demyelination observed in the posterior columns of three of the animals, however, does seem to be of some importance inasmuch as it was present only in the animals which survived the longest. By way of interpretation of this finding it could be supposed that a sufficient length of time is required for spinal changes of this type to occur. The time factor in this connection has already been shown to be an important one in the earlier study (3). But there must be another, more directly responsible factor for these degenerative changes and that, apparently, is the absence of vitamin B_2(G).

Goldberger and his associates assumed that the essential pellagra-preventive (P-P) factor lacking in the diets they employed was vitamin B_2. These diets, however, lack or are partially deficient in other essential materials. They may be inadequate in some mineral substances, and are recognized to be low in protein. The absence of these factors probably plays an important rôle in the production of the fulminating, rapidly progressive condition of black tongue, and it is this rapid progression of the disease that doubtless prevents the appearance of degenerative changes in the spinal cord produced by the concomitant deficiency of vitamin B_2(G).

The findings in these animals do not suggest any failure of gastric secretion, with respect to either acid or pepsin, and certainly there is no lack of salivary secretion. The studies on the blood of the dogs surviving 64, 123 and 155 days respectively, do not reveal a macrocytic anemia; the changes found seem to be correlated most satisfactorily with repeated blood loss as a result of hemorrhagic diarrhea. The prompt response to ferric and ammonium citrate by an elevation of the total number of red blood cells and of hemoglobin, in the absence of other forms of therapy, would support this view. These results do not confirm those of Rhoads and Miller (7), who found evidence of a macrocytic anemia in dogs with chronic black tongue.

SUMMARY AND CONCLUSIONS

Six adult dogs maintained on diet number 195 of Goldberger and his associates developed the clinical picture of black tongue in from 64 to 155 days.

Dogs 70, 155 and 157 which lived for 123, 119 and 122 days, respectively, before coming to necropsy, showed early, slight degenerative changes of the medullary sheaths of the posterior columns of all levels of the spinal cords. These changes differ in degree but not in kind from those produced in dogs by diets deficient in vitamin B_2(G). Dogs 69, 74 and 142 which lived for 155, 64 and 83 days, respectively, on the same diet, showed no traces of any degenerative changes in the spinal cord. From this it is concluded that the length of time that the animals were maintained on this diet was an important factor in the production of this lesion. Dog 69, with a survival time of 155 days, is the only exception to this rule, and this animal did show demyelination of the peripheral nerves.

Studies of the gastric secretions and blood of these animals failed to substantiate the idea that the deficiency involved in this diet has any relation to the syndrome of combined system disease.

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WHAT CONSTITUTES A LETHAL REDUCTION OF TEMPERATURE?

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What constitutes a lethal reduction of temperature on exposure to cold is a question of considerable theoretical and practical interest. Every winter skaters and laborers break through the ice and are exposed to water at or near the freezing point. Following the Titanic disaster in April, 1912, the survivors in boats were taken on board and further rescues were expected of those in the sea. No such rescues were made for none survived. There would doubtless have been many survivors if the sea water had been about body temperature.

The literature on the question is rather meager. Schafer records low temperatures in hibernating animals, newborn pups and kittens, and adult dogs and cats (1). Hill and Macleod experimented with mice. They obtained considerable reductions in temperature by exposure to currents of moist air (2). There are some data on accidental exposure of drunkards to cold (1). Because of the meagerness of the literature and variability of the temperature recorded in man, it was considered important to investigate the question further.

METHODS. Our experiments were performed chiefly during the colder months of the year so that more pronounced results could be obtained. Before starting work on each group of rabbits (about ten or twelve in number) a fixed routine was followed. The room temperature and outside air were noted; water was allowed to run from the tap for a half-hour and its temperature recorded. Two metal tubs were filled with running water. Next the rabbits were marked for identification and their initial temperature recorded. Two rabbits were immersed in each tub, all necessary precautions being taken to permit normal respiration. Throughout the period of immersion a constant flow of water was kept going into the tubs to maintain a nearly uniform water temperature. The animals were observed carefully throughout to avoid needless discomfort.

Upon removal from the bath the rabbits were drained somewhat by squeezing the fur. Their temperature was taken and they were in most cases subjected to outdoor exposure on a window ledge. This exposure averaged about 17 minutes in the earlier series of 111 rabbits.

At the end of the outdoor exposure their temperature was again noted. They were rubbed dry and placed by a warm radiator where they had access to food and water. A Centigrade thermometer was used in this series: it had been checked against a standard furnished by the department of physics. The temperature of the animals was taken per rectum, the thermometer being inserted to a depth of at least 6 cm. since less than this would give considerable variations (3).

Results. In the first series of fifty animals which were immersed an average of 11½ minutes and which were exposed to outdoor temperature (average seven and eight-tenths below zero°C.) an average of 16.8 minutes, only one fatality occurred. This fatality was due to an accident. The lowest rectal temperature recorded was 24.5°C. In the next series of 60 rabbits (average immersion 11.6 minutes and outdoor exposure of 17.1 minutes) there were only three fatalities. In these three cases the rectal temperature was reduced to 16.3°, 20.8°, and 25.5°C. The average temperature of the water was 8.1°C. Thus in the 111 rabbits so exposed a mortality of only 2.7 per cent occurred. These results show that the exposure used was rarely lethal and further indicate that considerable variation in tolerance to short exposures to cold, and in the lethal temperature, probably obtains.

In a group of 24 animals the immersion period was extended to an average of twenty-one minutes. The average temperature of the water was 3.5°C. All of these rabbits died. Eight of them died without being subjected to outdoor exposure. The remainder were subjected to an average exposure of eleven minutes. In this group of 24 rabbits all but three had a rectal temperature of less than 19°C. The temperature in the three exceptions was 19.7°, 20.3° and 20.5°C. The lower temperature of the water together with the longer period of immersion accounts for the 100 per cent mortality, in contrast to the 2.7 per cent mortality observed in the first group of experiments. In subsequent investigations using water having an average temperature of 3.4°C., it was found, by varying the period of immersion, and after omitting the period of outdoor exposure, that the mortality seldom exceeded 50 per cent.

The average initial temperature of 126 readings was 39.42°C. The lowest temperature observed in the entire series was 12.9°C. which occurred in one of the heaviest rabbits (weight 2420 gm.). The immersion period was 41 minutes and the outdoor exposure 16 minutes. This animal, rather surprisingly, made a complete recovery.

Discussion. Our results strongly indicate that there is no definite temperature above which life is always preserved and below which death always results. There are perhaps a number of variants which may be responsible for lack of definite results. One of these is the time required for decreasing the temperature, another is the time of return toward the nor-

mal, and a third is the individual resistance of the rabbit. Bodily vigor, surface area, thickness of subcutaneous fat deposit, and character of fur, might each contribute to this resistance.

An approximate line of cleavage may be set at 19°C. or lower. In 61 instances (out of a total tabulation of 277) this temperature or a lower one was observed. In these 61 instances the mortality was 62.3 per cent. This is a much greater mortality than in the remaining 215, which shows only about 9 per cent deaths.

We would suggest a further subdivision into two groups: first those from 19 to 25°C. and second those from 25° to 39.42°C. In the first group there were 103 cases showing a mortality of 15.5 per cent while in the second group there were 113 showing a mortality of 2.7 per cent. This last figure takes no account of three deaths which were clearly due to other causes.

SUMMARY

On exposure to cold a definite lethal temperature (above which life is always preserved and below which death always occurs) cannot be stated. The reason for the variation is to be explained as due to other bodily factors which tend to maintain, to conserve, and to return to normal, the body temperature. Sixty-two per cent of the rabbits whose body temperature had been reduced to 19°C. died: 15.5 per cent of those whose body temperature had been reduced to from 19° to 25°C. died: and death came to only 2.7 per cent whose body temperature had been reduced to only 25°C. or above.

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STUDIES ON THE ACTION OF THE ALKALI IODIDES

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The demonstration of the importance of the element iodine for the normal function of the thyroid gland and its subsequent extensive use in the prevention of goitre has stimulated a number of investigations on the action of this element and its alkali salts on the mammalian organism.

Macela (1925) reported that the addition of 0.2 mgm. NaI daily to the diet of rats produced a greater increase in weight and more rapid sexual development than was observed in control animals. This was confirmed by Hanzlik, Talbot and Gibson (1928) and Kochman (1931) who concluded that it was due to a lowering of the basal metabolism. Mendel and Vickery (1930) reported that the daily administration of 1.1 mgm. KI to the diet of rats for 200 to 400 days had no effect on the weight of growing rats. Evvards and Culbertson (1925) reported that growing pigs receiving 0.01 per cent KI daily showed an average weight increase 10 per cent greater than the control animals.

Grey and Loeb (1928) reported that small amounts of I caused stimulation of the thyroid gland of the guinea pig during the first three or four weeks of administration, but after this time I caused depression of thyroid function. Rabinowitch (1930) using the number of mitotic figures as an index of activity, confirmed this finding in the guinea pig. Chouke (1930) found that small amounts of KI administered for periods of 10 to 30 days had no noticeable effect on the thyroid of the rat. Webster and Chesney (1928) reported that although Lugol's solution caused a slight reduction in the basal metabolic rate of rabbits, it produced no changes in the thyroid.

Cases of thyrotoxicosis in supposedly normal individuals as the result of excess iodine intake have been reported by Buchholz (1918), Scheick (1930), Herzfeld (1931), Holm (1931), Raab (1931) and Zimmermann (1931). All these reports are subject to the criticism that the functional condition of the thyroid previous to the iodine administration was not known.

The action of iodides on the gonads was reported by Jastram (1920) who states that alkali iodides in doses under 2.0 grams per day caused an

[1] Aided by a grant from the Henry Strong Denison Foundation.

increased connective tissue formation in the ovaries of puppies; and by Buchhelm (1932) who reported that small amounts of iodine cause arrested development of the seminal vesicles of mice, rats and guinea pigs, but no interference with normal spermatogenesis.

Massimo (1930) reports that rabbits given 100 to 200 mgm. of KI per kgm. for 7 to 25 days show hemorrhagic infiltrations between the myocardial fibers and round cell infiltrations beneath the endocardium.

Sgalitzer (1908) reported the acute effects of large doses of NaI. Subcutaneous administration of 1.2 gram of NaI per kgm. to rabbits caused death in a few days. At autopsy there was marked fatty degeneration of the liver, frequent pleural effusion and pulmonary edema. Smaller doses caused severe diarrhea, loss of weight, and inanition. A characteristic effect produced was a decrease in voluntary movements of the animal, paralysis of the hind limbs, and marked dyspnea. Prolonged administration of smaller amounts produced a tolerance to the drug. Wadi (1928), also using rabbits, reported that 0.5 gram KI per kgm. orally per day produced a gradual loss of weight. He also reports the development of tolerance to the drug. At autopsy there was no pleural effusion, but marked hyperemia and edema of the lungs. The testicles were normal; the thyroid showed marked flattening of the acinar epithelium, and both normal and KI fed animals showed frequent fatty degeneration of the liver. Shoemaker and Underhill (1932) also state that administration of 0.5 gram KI per kgm. orally caused death in 2 to 4 days. Chiari and Januschke (1910) reported that the intravenous injection of NaI in dogs caused marked pleural effusions, which was confirmed by Chu and How (1931) but denied by Gold (1928) who used twice the dose of iodide used by Chiari and Januschke.

These discrepancies in the literature as to the effect of the alkali iodides suggested further studies on their effects in the rabbit.

METHODS AND MATERIALS. Experiments on the effect of iodides on growth were carried out on litter-mate animals raised in this laboratory. The thyroids were examined at biopsy, and a section removed for histological examination 1 or 2 weeks before administration of KI. KI was given orally in small capsules or subcutaneously in isosmotic solution (2.55 per cent KI). Weight, appetite, and consistency of the feces were recorded daily. At the conclusion of the experiment the animals were killed by a blow on the head and autopsy was carried out at once. The thyroid and sections of the heart, lungs, kidneys, adrenals, liver and ovaries or testes were fixed in Zenker's or Bouin's fluid and stained with hematoxylin and eosin. The results of these experiments carried out on three litters are shown in figures 1 to 3.

The effect of KI given subcutaneously in large doses on adult animals was studied on 5 animals weighing between 2000–2200 grams. Five

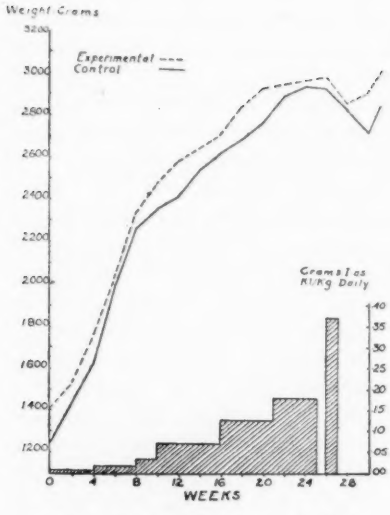

Fig. 1. Experimental, 2-control, 2 animals

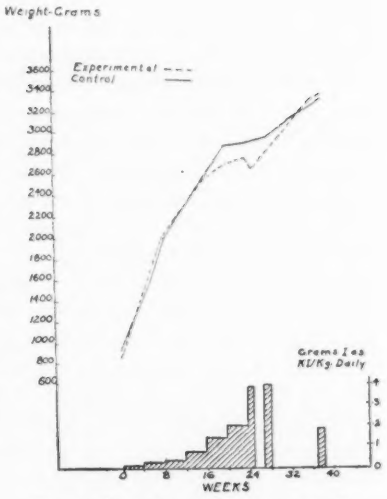

Fig. 2. Experimental, 4-control, 2 animals

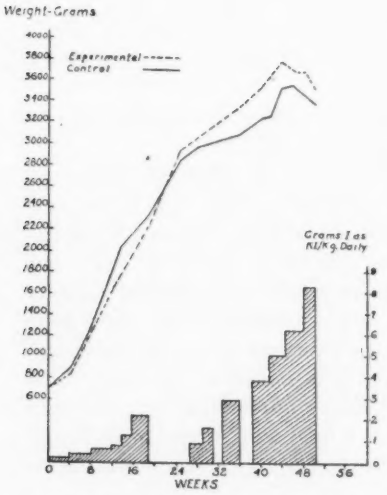

Fig. 3. Experimental, 5-control, 3 animals

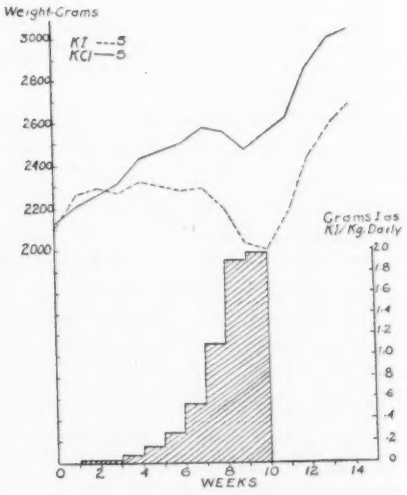

Fig. 4

control animals received an equivalent dose of KCl. The results are shown in figure 4.

The comparative effect of large doses of KI and NaI on young rabbits was studied on one litter, five animals receiving NaI and four KI subcutaneously. These results are shown in figure 5.

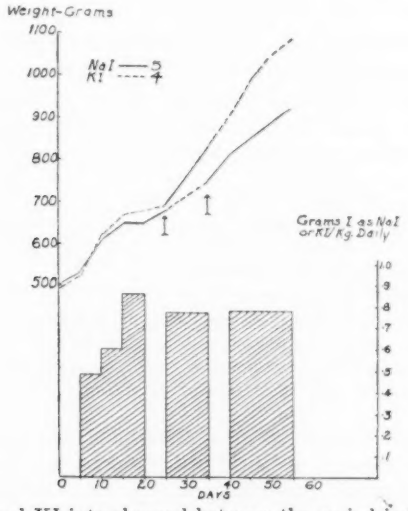

Fig. 5. NaI and KI interchanged between the period indicated by arrows

RESULTS AND DISCUSSION. *1. Growth.* The growth curves of the animals given KI orally (figs. 1 and 2) are not significantly different from those fed only on the stock diet. Diarrhea occurred occasionally in both experimental and control animals, with about equal frequency in both groups. The animals fed KI showed a polyuria and increased water intake, but in all other respects appeared to be normal. At autopsy there were no macroscopic changes in any organ except the thyroid, which was very hard and avascular. Pleural effusion or macroscopic evidence of fatty degeneration of the liver was never found in these animals.

Because of the difficulty experienced in giving KI quantitatively by mouth, another litter was given KI subcutaneously (2.55 per cent KI-fig. 3.). This concentration caused no cutaneous lesions even after months of injection. To rule out the potassium ion as the cause of any observed effects, the control animals received an equal volume of isomolar KCl. Both the experimental and control animals showed a marked polyuria and occasional diarrhea. When the dose of KI exceeded 0.5 gram per kgm. body weight, the characteristic flaccidity and paralysis of the hind limbs

observed by Sgalitzer appeared immediately following injection, but passed off within 20 to 30 minutes. This effect never appeared in the animals injected with KCl, and is definitely an action of the iodide ion. Two animals, after having received KI for 2 and 8 months respectively, went into severe convulsions, showed marked dyspnea, and died within 2 to 3 minutes following an injection of KI. This was most probably due to an accidental injection of some of the solution into a subcutaneous vein and is an effect of the potassium ion. Intravenous injection of NaI in twenty rabbits never produced these convulsions. Intravenous injection of small amounts of KI or KCl, however, always produced the typical convulsions and dyspnea which ended fatally unless artificial respiration was administered. The following protocol will illustrate a typical experiment.

Rabbit w 12. Male, 3150 grams. May 14, 1933.

2:00 p.m. 255 mgm. KI intravenously. Animal went into severe convulsions at once; marked dyspnea, exophthalmos, cardiac arrest. Artificial respiration administered. Recovery.
2:06 p.m. Animal walking about normally.
2:10 p.m. Injected 232 mgm. NaI intravenously. No effect.
2:18 p.m. Injected 232 mgm. NaI intravenously. No effect.
2:21 p.m. Injected 116 mgm. KCl intravenously. Immediate convulsions, dyspnea; animal died in spite of artificial respiration.

The results obtained on this limited number of animals with the massive doses of KI employed cannot be considered as evidence either for or against an accelerating effect of KI on growth. The fact that such large doses can be given over so long a time period (26–48 weeks), however, does indicate that growing rabbits develop a high tolerance to KI. The terminal doses in these experiments were larger than those reported by Sgalitzer (1908), Wadi (1928) and Shoemaker and Underhill (1932) as fatal when given as initial doses.

2. Effects of large amounts of KI given subcutaneously in adult rabbits. The great tolerance shown by young rabbits to increasing doses of KI suggested an experiment on the development of tolerance in adult animals (fig. 4). In these animals, KI in doses exceeding 1 gram I as KI per kgm. body weight caused a prompt loss of weight, severe diarrhea, and marked loss of appetite. The characteristic hind-limb paralysis was also observed and became more marked as the amount of KI was increased. That this weight loss, anorexia and paralysis are not due to the large amounts of potassium given is shown by the complete absence of these symptoms in the KCl-injected animals. At autopsy, none of these animals showed pleural effusion. Microscopically, definite fatty degeneration was observed in the livers of all animals injected with KI. Although these doses were markedly toxic, they are much larger than those reported as being rapidly fatal by the workers previously mentioned.

3. The comparative effect of large doses of NaI and KI. The greater toxicity of the potassium ion would suggest that NaI would be less toxic than KI when given subcutaneously in amounts equal in iodine content. This was investigated on one litter (fig. 5). Five animals received NaI (2.32 per cent), and four received KI (2.55 per cent). Both NaI and KI in the doses given caused profound depression of the animals, severe diarrhea, polyuria, and dyspnea for a variable time following the injection. One animal in each group died after a few days of severe diarrhea. The typical paralysis observed following KI was also observed following NaI. Although these doses caused marked symptoms of toxicity, growth occurred in both groups. KI did not appear to be more toxic than NaI as judged by the growth curves and the behavior of the animals.

4. Pathological changes. The most consistent and striking effect of prolonged iodine administration is shown by the thyroid gland. Macroscopically, this appears hard and avascular. Histologically, the thyroid shows marked flattening of the acinar epithelium and deposition of a large amount of hard, deeply staining colloid in the alveoli. The frequency of pleural effusions reported by Sgalitzer was not confirmed. Definite fatty degeneration was found in the livers of both adult and growing animals receiving large initial doses of iodide, but not in the livers of animals in the growth experiments who had developed a marked tolerance to the iodide. Massimo's finding (1930) of hemorrhagic infiltrations between the myocardial fibers and round cell infiltration beneath the endocardium after KI administration was observed in eight of the twenty hearts studied. Jastram's report (1920) that iodide caused an increased connective tissue formation in the ovaries could not be confirmed in the rabbit ovaries studied.

CONCLUSIONS

1. The oral or subcutaneous administration of KI to growing rabbits in progressively increasing doses up to 0.5 gram per kgm. body weight when given orally or 1.0 gram per kgm. body weight when given subcutaneously, does not alter appreciably the rate of growth.

2. Prolonged administration of KI leads to the development of a marked tolerance to the substance, so that doses which on first injection would be fatal can be given without producing toxic symptoms.

3. The acute effects following a large dose of the iodide ion are flaccidity and paralysis of the skeletal musculature, particularly of the hind limbs, and dyspnea.

4. The most striking histological change produced by prolonged iodide administration is a marked flattening of the epithelium of the thyroid acini and deposition of large amounts of colloid in the alveoli.

The author wishes to express his thanks to Dr. A. J. Carlson for his interest and advice during the course of this work.

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THE CALCIUM CONTENT OF FROG NERVE

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Calcium constitutes a large fraction of the total cation content of nerve tissue according to figures that are found in the literature. Such a proportion of calcium seems to be one essential difference between muscle and nerve tissue. Roeder (1930) investigated the salt content of resting and stimulated frog nerves and reported that resting nerves contained 0.435 to 1.52 per cent of calcium on the basis of dry weight, or 5.0 to 18.9 milli-equivalents of calcium per 100 grams of moist tissue. The calcium and potassium of stimulated nerve was increased toward the muscle end and decreased toward the other or stimulated end. He used a radiometric method which had been developed by Ehrenberg (1925). We have not been able to find any other figures on the calcium content of amphibian nerve.

Various values have been reported for the calcium content of nerves of mammals. Weil (1914) reported that human peripheral nerve contained 0.198 gram of calcium per 1000 grams tissue, wet weight, or 0.986 milli-equivalent of calcium per 100 grams of tissue. Kraus, Wollheim, and Zondek (1924) found for the vagus nerve of dogs values of 0.085, 0.2 and 0.27 per cent wet weight; for dog ischiadic nerves, 0.148, 0.264 and 0.215 per cent wet weight, or 4.23 to 13.4 milli-equivalents per 100 grams nerve. Simon and Szelôczey (1928) found in general large variations of calcium in rabbit nerves. Some of the figures given vary several hundred per cent from their mean value. Their maximal values go as high as 1.92 per cent wet weight.

More recently Hayasi (1931) has determined the calcium content of the tongue nerve of some marine mammals ("*Seiwal*"), using the oxalate precipitation and potassium permanganate titration method of analysis. The nerves contained 0.019 to 0.035 per cent calcium, wet weight, or 0.99 to 1.75 milli-equivalents of calcium per 100 grams of tissue.

The figures reported on calcium in nerve thus show large variations. However, there has been no attempt to locate the calcium in nerve tissue, or to determine the relation between the calcium inside the nerve and the calcium of the fluids with which the tissue is in equilibrium. The present investigation presents additional analyses for calcium in frog nerve and offers some evidence as to the probable location of the calcium in the tissue.

The experimental procedure in general has been to immerse one set of nerves of matched pairs in Ringer's solutions of different calcium concentration for a sufficient length of time to insure equilibrium between the tissue and the surrounding solution. The tissue was then analyzed for calcium. The volume of solution in which the nerves were soaked was large in comparison with the tissue sample used (50–100 cc. of solution to 100 mgm. of nerve). The other set of nerves was used as a control and was analyzed either immediately after dissection or was soaked for corresponding lengths of time in normal Ringer's or some other desired solution and then analyzed for calcium. The solutions were oxygenated at 22°C. for the duration of the experiment. The osmotic pressure was maintained by variation of sodium chloride concentration corresponding to any variations which were made in other components of the Ringer's solution. In other sets of experiments, the outside potassium, pH, or phosphate concentrations were varied. No especial regard has been given to restricting the variations within physiological ranges.

Since the tissue samples were small and of low calcium content, it was thought that a colorimetric method of calcium analysis would be preferable. The method which Roe and Kahn (1929) developed for blood serum calcium was adopted. The method was so modified that it could be used with tissues other than blood and was further adapted so that it could be used with small samples of tissue containing a few hundredths of a milligram of calcium.

The nerve tissue was weighed, placed in platinum crucibles and dry-ashed overnight in an oven at a temperature gradually reaching but not exceeding 600°C. The white ash was taken up in dilute HCl, transferred to a centrifuge tube, and the solution made slightly alkaline with 10 per cent sodium hydroxide. The solution was allowed to stand for 5 minutes for the precipitation of magnesium hydroxide. Additional alkali was then added, excess phosphate added, and the solution allowed to stand for 1 hour to insure complete precipitation of calcium phosphate. The remainder of the method was as described by Roe and Kahn. The precipitate was washed, molybdic-sulfuric acid reagent added, and the phosphomolybdate reduced with aminonaphtholsulfonic acid. The resulting blue color was compared with that developed by similar treatment of a standard solution of KH_2PO_4. Samples of tissue containing 0.02 mgm. of calcium could be analyzed to an accuracy of 0.0006 mgm.

Results. The sciatic nerves of leopard frogs (winter frogs) were dissected and analyzed immediately for calcium. Frogs of both sexes were used since no consistent sexual difference was noted. A sample of three nerves was necessary for each determination. The values for calcium content ranged from 0.560 to 0.905 milli-equivalent calcium per cent. The mean of 28 determinations was 0.735 milli-equivalent of calcium per 100

grams moist tissue, with a standard deviation of ± 0.0937 milli-equivalent per 100 grams. In twenty-one of the determinations, matched nerves, i.e., the three nerves of the other legs of each group of frogs, were analyzed to determine the extent of variation between symmetrical groups. The extreme variation was 0.122 milli-equivalent per cent with an arithmetical mean variation between the matched nerves of 0.0347 milli-equivalent of calcium per 100 grams of moist tissue. The probable error of the mean variation was ± 0.0186 milli-equivalent per cent. It was essential that this be known in order that one of the matched nerves could be used as a control and the other as experimental.

It was surprising to find such low values for the calcium content. The values reported by Roeder (1930) are so much greater than the ones presented in this report that comparison is almost impossible.

It is of interest to include some figures for the calcium content of non-medullated nerves. Dr. A. H. Hegnauer collected some crab and lobster nerves at Woods Hole in August, 1933 and brought them back to the laboratory. Two determinations on crab nerves showed calcium contents of 1.42 and 1.28 milli-equivalents per 100 grams moist weight. Values for lobster nerves were 1.42, 1.09 and 1.3 milli-equivalents per cent. However, the body fluids of these crustaceans contain much more calcium than the blood plasma of frogs. The body fluid of the crab contains 2.79 milli-equivalents per 100 cc. and that of the lobster has a somewhat higher concentration.

In one series of experiments the nerves of frogs were soaked for 5 hours in Ringer's solution with calcium concentrations from 0 to 4.35 milli-equivalent per 100 cc. The phosphate content was 4.1 mgm. per cent phosphorus. During the course of the experiment the pH varied from 7.40 to about 7.00. After 5 hours' soaking the nerves were weighed and analyzed for calcium. Figure 1 shows the results obtained. The values for calcium inside the nerves after soaking are expressed on the basis of initial weight. The straight line drawn across the graph and marked "av. initial" represents the amount of calcium found in freshly dissected nerve and also represents the average calcium content of the control nerves. I have made a few analyses for calcium in frog plasma finding on the average 0.4 milli-equivalent per cent. This value must be approximate since in most samples a calcium precipitate was thrown down when the cells were separated by centrifuging. However, deWaard (cited by Hamburger and Brinkman, 1918) reported the following values for plasma from winter frogs: 0.23, 0.78, 0.3 and 0.3 milli-equivalent per 100 cc. The arrows indicate the calcium concentration of normal Ringer's solution and of blood plasma.

There are several points of interest in this graph. At outside concentrations of 0.4 milli-equivalent per 100 cc., the nerves neither gain nor

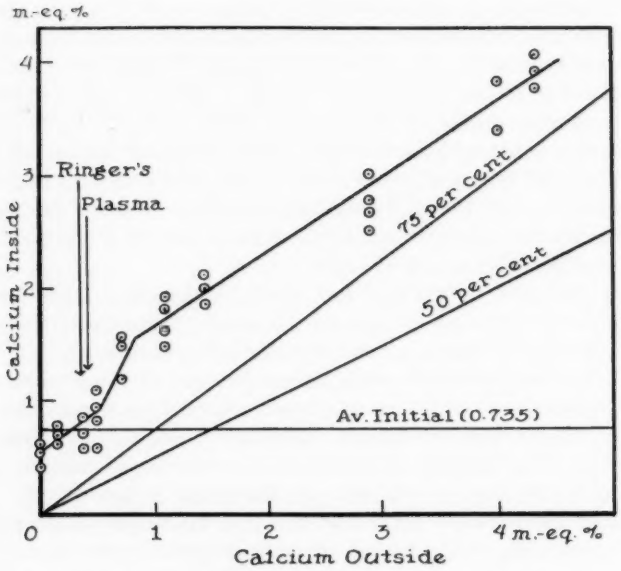

Fig. 1. The calcium content of frog nerves soaked for 5 hours in Ringer's solutions of varying calcium concentration. The lines marked 50 and 75 per cent indicate the variation in calcium content in the nerve if those fractions of the total nerve contained calcium at the same concentration as the outside solution. For fuller description see text.

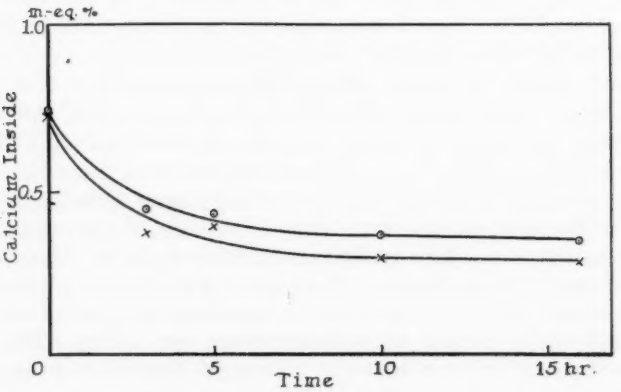

Fig. 2. The calcium content of frog nerves after soaking in isotonic solutions of NaCl and glucose. Glucose, -o-; NaCl, -x-.

lose calcium during soaking. Thus the normal calcium equilibrium is not disturbed by dissection.

In calcium-free Ringer's solution even after 5 hours' soaking 40 to 50 per cent of the calcium is still held within the nerve. The amount that diffuses out of the nerve is 0.2 to 0.35 milli-equivalent per 100 grams (initial weight). Figure 2 gives additional data on the diffusible and non-diffusible calcium of the nerve. Matched nerves, one set of which was soaked in isotonic sodium chloride solution, and the other in isotonic glucose solution, were used. Calcium diffuses out rapidly in the first 3 hours, but levels off after about 5 hours of soaking. The calcium diffusing out during the subsequent 10 to 12 hours is almost negligible. After 5 hours' soaking, 50 to 60 per cent of the calcium originally present still remains in the nerve. In this respect there seems to be little difference whether the soaking medium is NaCl or glucose. However, the nerves soaked in glucose swell to varying extents, showing a gain in weight of 23 to 40 per cent, while those in sodium chloride do not vary from the initial weight more than they do on soaking in normal Ringer's solution. This is probably explained by the penetration of glucose into the nerve fibres without any corresponding loss of electrolytes, thus increasing the osmotic pressure inside and causing swelling. In nerves in NaCl, however, the inside osmotic pressure is not affected so greatly.

A third interesting feature of the curve in figure 1 is the sudden increase in nerve calcium (0.5 m.-eq. per cent) which occurs when the outside concentration is raised from 0.5 to 1.0 milli-equivalent per 100 cc. At higher concentrations the graph continues at a slope approximately the same as the slope before the jump. A similar increase in calcium occurs even when no phosphate is present in the outside solution, but is of smaller magnitude, not exceeding 0.3 milli-equivalent calcium per 100 grams. With increase of outside phosphate and the appearance of an appreciable precipitate of calcium phosphate in the solution the magnitude of the jump increases, and under proper conditions may reach very high values (see table 3).

Included in figure 1 are diagonals drawn to represent the calcium inside the nerve if 50 per cent and 75 per cent, respectively, of the nerve tissue had the same calcium concentration as the outside solution. About 75 per cent of the total nerve weight is made up of water. Since the slope of the experimental points approximates that of the 50 per cent line it may perhaps be concluded that only about 50 to 55 per cent of the nerve is involved in the calcium diffusion process, i.e., that this fraction of the nerve weight contains calcium in a concentration which is always equal to that in the solutions. Frog nerve when soaked in normal Ringer's solution swells somewhat, gaining an average of 4 per cent in weight. In a series of experiments presented in figure 1 the swelling was not appreciably different from the average in normal Ringer's solution.

The fraction of the nerve involved in the calcium exchange is affected very little by a ten-fold variation of the potassium concentration of the outside solution. In figure 3 are presented data from experiments in which the calcium concentration of the outside solution was kept constant and the outside potassium concentration varied from 0.134 to 1.32 milli-equivalent per 100 cc. After soaking for 5 hours the nerves were analyzed for calcium. Controls were soaked in normal Ringer for the same period of time.

A second series of experiments was carried out in which the outside calcium concentration was varied but the ratio of potassium to calcium was kept constant over the whole range. The results have not been included

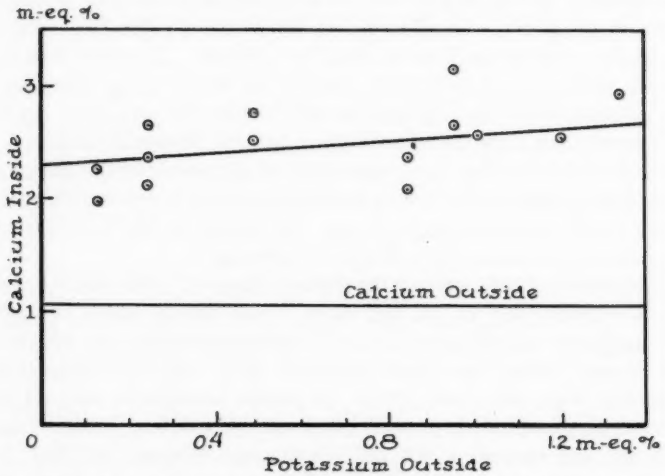

Fig. 3. The effect of varying outside potassium concentration on the calcium content of frog nerve.

in the present report, but in general there were no alterations in the slope of the curves. The magnitude of the sudden increase in calcium inside due to calcium precipitation in the nerve was slightly larger, but the variation did not seem to be significant.

Nerves were soaked for 5 hours in calcium-free Ringer's solutions of varying pH. The solutions were buffered with phosphates at pH 5.64, 6.6, 7.38 and 8.5. The results are shown in table 1. Changing the pH over a wide range had very little effect on the amount of calcium diffusing out of the nerve. The slightly lower calcium content in the more acid solutions is scarcely a significant difference.

Figure 4 gives the results of some additional experiments on pH. This series was a duplicate of the series presented in figure 1 except that the

solutions were buffered at a pH of 5.64 with phosphate buffer. A more acid solution would tend to prevent the precipitation of calcium phosphate outside until higher calcium concentrations were reached. Such a shift in the precipitation outside would shift the break in the curve toward the higher concentrations of calcium. This expected shift is present but almost insignificant in amount. The slope appears to be steeper than the slope of the graph in figure 1 at low concentrations of calcium but becomes less steep at higher concentrations. The low pH may produce some injury affecting the permeability of the cell membrane thus involving a greater fraction of the nerve in the calcium diffusion.

Two series of experiments were carried out with phosphate. In the first series the outside phosphate concentration was varied, the calcium concentration and the pH remaining constant. In the second, the phosphate

TABLE 1

The calcium content of frog nerve soaked for 5 hours in calcium-free Ringer's solution at different hydrogen ion concentrations

(The calcium values are given on the basis of the initial weight)

pH	CONTROL NERVES* Ca CONTENT	EXPERIMENTAL NERVES	
		Gain in weight	Ca content
	m.-eq. per cent	*per cent*	*m.-eq. per cent*
5.64	0.695	0.39	0.374
6.60	0.705	5.0	0.358
7.38	0.730	4.0	0.407
8.50	0.745	1.0	0.394

* Freshly dissected.

concentration and pH were kept constant and the outside calcium concentration was varied. The difference between the latter experiments and those given in figure 1 is that the phosphate concentration was increased four times. The results of two experiments are reported in table 2. Of particular interest is the increase of calcium in the nerve with increase of phosphate concentration in the outside solution and the sudden large jump in calcium content of nerve which occurs in both experiments at the point where visible heavy precipitation in the outside solution first appears. At concentrations lower than the one at which the large increase occurs, there is no appreciable swelling of the nerve beyond that which occurs in normal Ringer's solution. However, the gain in weight when the calcium content inside jumps indicates a gain in osmotic pressure of 20 per cent, although the decrease in osmotic pressure outside as a result of the precipitation of calcium phosphate accounts for 5 per cent of the gain. The 85 millimols increase in calcium would result in an increase

in osmotic pressure of three or four times that observed. Crystalline calcium phosphates seems to be injurious to the cells, which with altered permeability may allow anions to enter with the cations. The cations

TABLE 2

The calcium content of frog nerve soaked for 5 hours in Ringer's solution with different concentrations of phosphate and calcium

Ca OUTSIDE	P OUTSIDE	pH	GAIN IN WEIGHT	Ca INSIDE
m.-eq. per cent	*m.-eq. per cent*		*per cent*	*m.-eq. per cent*
1.07	0.0	6.4	3.0	1.95
1.07	0.396	6.4	4.2	2.5
1.07	0.792	6.4	4.0	3.07
1.07	0.19	6.4	4.7	3.58
1.07	1.58	6.4	15.0	21.2
0.00	1.58	7.38	3.5	0.730
0.119	1.58	7.38	3.5	1.06
0.36	1.58	7.38	5.5	1.145
0.715	1.58	7.38	4.2	1.71
1.07	1.58	7.38	10.0	13.9
1.44	1.58	7.38	20.0	22.9

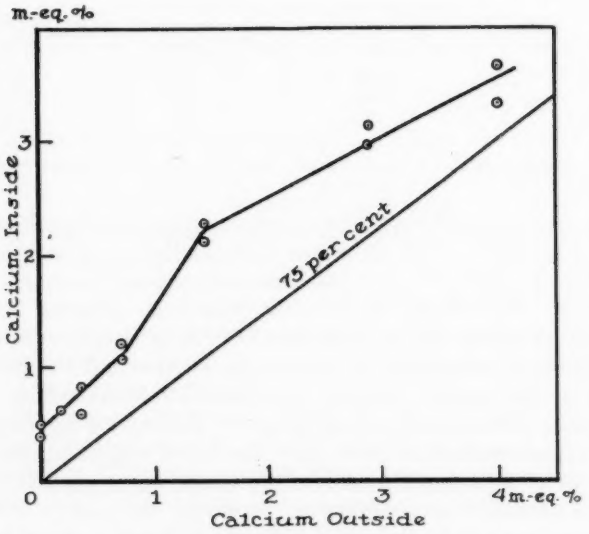

Fig. 4. The calcium content of frog nerves soaked in Ringer's solution of varying calcium concentration (pH, 5.64; P, 1.58 m.-eq. per cent). The straight line indicates the variation in calcium content of the nerves if 75 per cent had a calcium concentration equal to that of the outside solution.

concerned may be other than calcium ions since calcium is precipitating out.

Discussion. The foregoing experiments lead to the conclusion that about 55 per cent of the nerve is involved in the exchange of calcium between the tissue and the surrounding solution. Fenn and Cobb (1934) have been led to conclude that all the chloride and most of the sodium of the nerve is present in 50 per cent of the nerve when it is in equilibrium with the sodium chloride of blood plasma. Potassium appears to be in high concentration in the remainder of the tissue. They have thus divided the nerve into two regions, a "sodium chloride space" which includes 50 per cent of the nerve and is considered to be extracellular spaces. The remainder, the "potassium space" is considered to be the cellular spaces of the nerve tissue.

Calcium is probably present in both the "sodium chloride space" and the "potassium space." The water of the extracellular space is presumably in equilibrium with the surrounding solution and contains electrolytes which are in the same concentration as are those of the outside solution. The slope of the curve in figure 1 suggests that this assumption is correct. Since the slope is relatively constant at quite different concentrations of calcium in the outside solution there must be very little exchange between the calcium of the extracellular spaces and that of the cellular spaces. When the nerve tissue is in equilibrium with blood plasma there appears to be a considerably higher concentration of calcium inside the cells than in the extracellular spaces. The extracellular water has a calcium content of 0.22 to 0.235 milli-equivalent (fig. 1). This would involve about 50 grams of nerve water if we consider the calcium concentration to be the same as that of blood plasma. The 0.5 milli-equivalent of calcium located presumably inside the nerve cells must be in the remaining 25 grams of nerve water. The calcium concentration inside would be therefore approximately four times as concentrated as that in the extracellular fluids. The data presented in figure 2 show that a small fraction of the calcium inside diffuses out when the nerves are soaked in isotonic NaCl and glucose solutions. Probably these solutions injure the cells slightly.

The magnitude of the sudden increase in calcium content inside the nerve (fig. 1) and the outside calcium concentration at which this jump occurs depends to some extent on the conditions which govern the precipitation of calcium phosphate in the outside solutions. The increase may be due to precipitation of calcium phosphate inside the nerve. With no outside phosphate it is possible that the calcium precipitates with phosphate from inside the nerve cell. Gerard and Wallen (1929) showed that frog nerve contains 0.5 to 1.0 milli-equivalent per cent of inorganic phosphorus. Most of the phosphate is located inside the cells and is at least equivalent to the 0.3 to 0.5 milli-equivalent of calcium which seems to be precipitated

in the nerve. There was no significant change in the carbon dioxide combining power of the tissue after soaking in phosphate-free solutions containing calcium in concentrations just above and below that necessary for the precipitation of calcium inside. Hence, if inorganic phosphate is precipitated it has no effect on the buffering power of the tissue.

SUMMARY

1. The sciatic nerve of winter frogs contains an average of 0.735 ± 0.0937 milli-equivalent of calcium per 100 grams wet weight. A few figures for non-medullated nerves of crabs and lobsters are included.

2. When excised frog nerve is immersed in Ringer's solution of varying calcium concentrations until equilibrium between the calcium inside the nerve and the calcium outside is attained, the changes in the calcium of the nerve are such as to indicate that 55 per cent of the nerve is involved in the diffusion process. Evidence is presented that this portion of the nerve represents extracellular spaces. When the outside solution contains no calcium, 60 per cent of the calcium of the nerve does not diffuse out.

3. With sufficiently high concentrations of phosphate or of calcium in the outside solution, there is a sudden increase in the nerve calcium. The concentration at which this occurs seems to depend on the same factors as does the precipitation of calcium phosphate in the outside solution. At a constant outside calcium concentration the magnitude of the increase depends somewhat on the phosphate present outside. Precipitation of calcium phosphate somewhere in the nerve is suggested. This occurs even though there is no phosphate in the outside solution.

4. Varying the potassium concentration or the pH of the outside solution appears to affect the calcium diffusion very little. A more acid medium tends to involve a greater proportion of the nerve in the diffusion process.

I wish to express my appreciation to Dr. W. O. Fenn for his generous suggestions and directions during the course of these experiments.

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THE PERMEABILITY OF THE BLOOD CAPILLARIES TO LIPOIDS

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In connection with recent studies concerning the composition of lymph and the permeability of the blood capillaries (Drinker and Field, 1933), the question has arisen as to whether or not the normal capillary wall is permeable to the lipoid constituents of blood. Accordingly a series of experiments was undertaken to investigate this point. The method used has been that of determining simultaneously the total fat and cholesterol content of the blood plasma and cervical lymph of a dog before and after the production of hyperlipemia.

EXPERIMENTAL PROCEDURE. Dogs were used throughout. For the most part, animals which had fasted only overnight (16 to 18 hours) were used; in experiment 5 a dog which had fasted for 36 hours was used. To secure anesthesia, a 5 per cent solution of nembutal was injected intraperitoneally in approximate dosage of 40 mgm. per kilogram of body weight. Isolation of a cervical lymphatic on each side of the neck was carried out and the vessels cannulated as far caudally as possible in an attempt to secure lymphatic drainage not only from the head and neck but also from the upper extremities. During the course of these operative procedures, 200 to 250 cc. of physiological salt solution were injected intravenously to insure adequate hydration of tissues.

At the beginning of the series of experiments it was hoped that by the giving of a meal of cream or olive oil to a fasting dog, a satisfactory hyperlipemia could be regularly produced. This proved not to be the case. The rise in blood fat following a standard fat meal was erratic and varied both in degree and in time of occurrence from dog to dog. This was due in part probably to variability in the time required for passage out of the stomach and for digestion. Consequently the intravenous injection of fat was employed to produce hyperlipemia. At first, use was made of chyle collected from the thoracic duct of one or two dogs which had been given cream or olive oil. In the last two experiments a fat emulsion[1] (olive oil

[1] Kindly made and supplied by Mead Johnson & Co., Evansville, Indiana, whose generosity and coöperation we gratefully acknowledge.

and lecithin) which could be injected intravenously was used. This could be given in amounts as large as 460 cc. without apparent injury to the animal. It was never possible to obtain in sufficient quantities chyle with a fat content over 4.6 per cent; one fat emulsion, on the other hand, contained 16.8 per cent fat.

After both cervical lymphatics had been cannulated a control sample of lymph (slightly over 2 cc.) was collected and control specimens of blood secured. The collection of lymph was aided by downward massage of the neck of the animal. Following this, the chyle or fat emulsion was slowly injected intravenously. As much as 539 cc. of chyle or 460 cc. of the fat emulsion were injected without evident untoward reaction or change in blood pressure as indicated by a record made from the femoral artery. Samples of blood were withdrawn from the jugular veins at frequent intervals and lymph was collected continuously, fresh tubes being exchanged when quantities of slightly over 2 cc. had been obtained. At the height of the lymph flow, 2 cc. could be collected in 10 to 30 minutes. Potassium oxalate was used to prevent clotting of blood and lymph.

CHEMICAL METHODS. With the slight modifications indicated below, Bloor's colorimetric method for total cholesterol and his dichromate oxidation method for the determination of total lipid (Bloor, 1928) were used. Analyses were made in duplicate.

In the process of saponification, the measured quantity of alcohol-ether filtrate was evaporated until the volume was reduced to 10 cc. or less *before* the sodium ethylate was added (Yasuda, 1931; Okey, 1930).

In the determination of cholesterol, the modification as to temperature suggested by Mirsky and Bruger (1932) was employed in the last three experiments.

In the determination of total lipid, the following minor variations were found helpful in securing accurate results. In preparation for the process of oxidation, the normal dichromate solution was added *before* the silver-sulphuric acid reagent to insure the optimum water-acid ratio for the initial oxidation of the fat and to avoid the danger of too rapid decomposition of the acid reagent. Oxidation was carried out for 90 minutes on a steam bath with the temperature just inside the bath regulated to 90°C. $\pm$ 2°. In the titration, 10 cc. of 15 per cent potassium iodide solution were used to insure excess of this reagent. As an indicator, an 0.5 per cent solution of potato starch prepared in saturated benzoic acid was found satisfactory.

RESULTS. In all, 9 complete experiments were carried out. Of these, 3 have been discarded because of early technical difficulties. One experiment is not reported because the amount of chyle injected was so small that no appreciable rise in blood fat was obtained. In these, however, the results showed the same tendency which was noted in the 5 experiments reported in detail below.

Experiment 1. September 14, 1933. Dog 1, male, 15.2 kgm., fasted approximately 16 hours. Anesthesia secured by nembutal given intraperitoneally at 9:20 a.m. Exposure of cervical lymphatics started at 9:40 a.m. and collection of lymph begun at 10:15. Between 9:50 and 10:10, 220 cc. physiological salt solution were injected intravenously.

A satisfactory increase in the value for blood fat was obtained, and following the injection this fell slowly toward the initial level (see table 1). There was no significant effect on the blood cholesterol and the lymph cholesterol remained remarkably constant throughout. An unmistakable rise in the fatty acid content of the lymph was obtained. It is interesting that an acceleration of the rate of flow of the lymph occurred at a time which later proved to be that of the highest fat concentration in the lymph. This lymphagogue effect was noted under similar conditions in one other experiment not reported in this paper.

A blood pressure tracing made from the femoral artery before, during, and after the injection of 450 cc. of chyle showed no significant alteration.

Experiment 2. October 25, 1933. Dog 2, fasted approximately 17 hours. Under anesthesia produced by nembutal injected intraperitoneally, the cervical lymphatics were cannulated and collection of lymph begun at 10:08 a.m. Between 9:50 and 9:55, 250 cc. of physiological salt solution were injected intravenously.

In this experiment (see table 1) although a large amount of chyle was injected, its fat content was only 2.2 per cent and only a small rise in blood fat was seen following the injection. Only a slight rise in the fat content of the lymph was obtained. The temporary decrease in the blood cholesterol value was probably due to dilution of the blood with the chyle injected. The same is true of the decrease in the protein content of the blood. This is reflected in a dilution effect on the lymph and it is possible that this masks a tendency of the lymph fatty acid to rise which was greater than that observed.

Experiment 3. November 24, 1933. Dog 3, male, 19 kgm., fasted approximately 16 hours. Anesthesia secured by nembutal given intraperitoneally at 9:10 a.m., exposure of cervical lymphatics started at 9:30 and collection of lymph begun at 10:00 a.m. Between 9:45 and 10:00, 225 cc. of physiological salt solution were given intravenously.

Despite the injection of a large amount of chyle (total amount of fat injected, 20.7 gm.) and the production of a satisfactory hyperlipemia, no noteworthy rise in lymph fatty acid was seen (see table 1). It will be noted that the initial value for fatty acid in lymph was high, 399 mgm. per 100 cc.; in the experiments in which we have found a rise in the lymph fatty acid following the creation of hyperlipemia, the initial (fasting) fat content of the lymph has been considerably lower. This is probably related to the nutritional state of the animal. As in previous experiments no change was noted in the cholesterol content of the blood or lymph.

Experiment 4. January 18, 1934. Dog 4, male, 21.7 kgm., fasted approximately 17 hours. Under anesthesia produced by intraperitoneal injection of nembutal the cervical lymphatics were cannulated. Between 9:45 and 10:05 a.m., 250 cc. of physiological salt solution were injected intravenously. At 10:15, collection of lymph was begun.

As may be seen in table 1, there was a slight but definite increase in the total lipid content of the peripheral lymph following the production of the hyperlipemia. Since the fat emulsion contained an appreciable amount of cholesterol (206 mgm. per 100 cc.), the injection produced a rise in the cholesterol of the blood plasma of over 70 mgm. per 100 cc. It is noteworthy that this level of blood cholesterol was maintained throughout the remainder of the experiment, a matter of 4 hours, and no increase in the lymph cholesterol was obtained.

TABLE 1

Changes in the lipoids of cervical lymph accompanying hyperlipemia

NUMBER OF EXPERIMENT	TIME	BLOOD PLASMA			CERVICAL LYMPH			REMARKS
		Cholesterol	Fatty acids	Total lipid	Cholesterol	Fatty acids	Total lipid	
	a.m.	*mgm./100 cc.*						
1	10:23	153	411	578				Rectal temp. 101°F. at 10:35.
	10:55	153	418	582	67	203	276	
	11:03–11:30	Intravenous injection of 450 cc. chyle (total lipid content 4.3 gm. per 100 cc.)						
	11:37	144	1667	1978	63	258	331	
	11:58				67	287	359	Lymph flow is rapid.
	p.m.							
	12:02	148	1358	1682	64			
	12:13		1084	1380	63	354	423	
	12:23				62	322	388	Rate of lymph flow is slower.
	12:46	145	804	962	61			
	1:04	139	734	886	60	330	398	
	1:35	136	506	653	64	334	405	
	2:08	140	458	611	60	316	381	Rectal temp. 102.1°F. at 2:25.
	2:52	148	455	618	65	283	355	
	a.m.							
2	10:22	106	487	602				Blood plasma protein 6.21 gm./100 cc.
	11:40	126	506	643	50	371	426	Cervical lymph protein 5.03 gm./100 cc.
	11:45–11:58	Intravenous injection of 539 cc. chyle (total lipid content 2.2 gm. per 100 cc.)						
	p.m.							
	12:03	83	732	822				Blood plasma protein 5.57 gm./100 cc.
	12:10	88	594	697				Rectal temp. 102.8°F. at 12:08.
	12:34	96	497	602				Blood plasma protein 5.78 gm./100 cc.
	12:40				53			Cervical lymph protein 4.73 gm./100 cc.
	1:12	105	468	584	47	382	435	Cervical lymph protein 4.72 gm./100 cc.
	1:58	105	484	595	46	396	447	Blood plasma protein 5.67 gm./100 cc.
	2:42		455	561	52	332	388	Cervical lymph protein 4.57 gm./100 cc.
	3:08	106	425	541	52	327	383	
	3:45	106	411	536	54	310	368	Blood plasma protein 5.76 gm./100 cc.
								Cervical lymph protein 4.29 gm./100 cc.
	a.m.							
3	10:18	159	579	753				
	10:52	159	598	768				
	11:00				76	399	484	
	11:00:11:15	Intravenous injection of 450 cc. chyle (total lipid content 4.6 gm. per 100 cc.)						
	11:17	158	1659	1830				
	11:34	156	1412	1581	75	346	425	Rectal temp. 100°F. at 11:52.
	12:00	158	1393	1565	77	359	442	

TABLE 1—*Concluded*

NUMBER OF EXPERIMENT	TIME	BLOOD PLASMA			CERVICAL LYMPH			REMARKS
		Cholesterol	Fatty acids	Total lipid	Cholesterol	Fatty acids	Total lipid	
	p.m.	*mgm./100 cc.*						
	12:23	158	1156	1328	69	357	432	
	12:53	159	1157	1328	74	356	436	
	1:28	160	986	1159	77	343	425	
	2:15	159	730	901	73	370	445	Rectal temp. 101.6°F. at 2:30.
	2:48	159	627	834	75	279	361	
	3:04	156	633	838	71	421	499	
	3:28	156	529	700	71	411	488	
	3:50–4:06				71	339	417	
	4:05	159	533	704				
	a.m.							
4	10:30	165	426	601				
	11:25	168	441	620				
	p.m.							
	12:15				63	158	225	
	12:22–12:35	Intravenous injection of 460 cc. fat emulsion (total lipid content calculated as oleic acid, 6.0 gm. per 100 cc.; cholesterol content, 206 mgm. per 100 cc.)						
	12:43	241	1983	2244				
	1:18	242	1530	1795	55	162	221	
	2:15	239	940	1190	58	263	329	
	3:05	242	790	1062	56	182	243	
	3:15	225	698	945				
	4:35	238	681	937	61	222	295	
	a.m.							
5	10:22	170	414	599				
	10:59	173	415	604	72	202	279	
	11:05–11:15	Intravenous injection of 240 cc. fat emulsion (total lipid content calculated as oleic acid, 16.8 gm. per 100 cc.; cholesterol content, 425 mgm. per 100 cc.)						
	11:20	268	3197	3480				
	11:48	279	2873	3176	68	215	284	
	p.m.							
	12:18	290	2643	2956	67	218	291	
	1:08	281	2373	2680	69	209	285	
	1:50	271	2065	2368	66	205	277	
	2:30	265	1820	2100	64	243	313	
	3:22	281	1750	2059	63	246	315	
	4:00	270	1578	1870	63	241	309	
	4:35	270	1423	1712	63	218	286	

Experiment 5. April 18, 1934. Dog 5, male, fasted 36 hours. Anesthesia was secured by intraperitoneal injection of nembutal and exposure and cannulation of cervical lymphatics were carried out. Between 9:45 and 10:00, 225 cc. of physiological salt solution were injected intravenously. Collection of lymph began at 10:00 a.m.

In this experiment a satisfactory hyperlipemia was produced (see table 1). As in previous experiments, a slight rise (13 per cent) in the total lipid content of the cervical lymph was obtained. Although the fat injection produced a rise in the plasma cholesterol of over 100 mgm. per 100 cc., no increase was noted in the lymph cholesterol.

TABLE 2

Lipids of blood and cervical lymph of fasting dogs compared

DOG	BLOOD PLASMA			CERVICAL LYMPH		
	Cholesterol	Fatty acids	Total lipid	Cholesterol	Fatty acids	Total lipid
	mgm. per 100 cc.					
1	153	415	580	67	203	276
2	116	497	623	50	371	426
3	159	589	761	76	399	484
4	167	434	611	63	158	225
5	172	415	602	72	202	279
6	134			69		
7	104	336	448	33	139	183
8	113			34		
9	166	502	683	87	224	318
10	70	303	375	20		
11	148	451	621	41	214	250
Averages..	137	438	589	56	239	305

Note: All blood values represent the average content of two samples, one drawn at the beginning and one at the close of the collection of the corresponding lymph sample.

LIPIDS OF BLOOD AND LYMPH COMPARED. In table 2 are compiled the results obtained for the cholesterol and fatty acid content of lymph and blood plasma when secured simultaneously in the fasting state. The values are taken from experiments presented above and from certain experiments not reported in detail here. Each set of values is from a separate dog.

From this table it is evident that although the cholesterol content of the cervical lymph averaged only 41 per cent (56/137) of the cholesterol content of blood plasma drawn at the same time, the lymph total lipid values were relatively higher, being 52 per cent (305/589) of the corresponding blood values. These results are consistent with the findings already presented which were obtained after the production of hyperlipemia. They may be taken to indicate that the permeability of the blood capillaries to cholesterol is less than to other lipoid substances.

Discussion. Our results have varied somewhat from dog to dog, but it is fair to state that in general, following the intravenous injection of a large amount of fat-laden chyle or of a fat emulsion, there has occurred a slight rise in the fatty acid content of the cervical lymph. The height of the rise has taken place in from 40 minutes to over 4 hours from the time the injection of fat was finished; the average interval has been about 2 hours. In two experiments, it was noted that during the period of increase of the fat of the lymph there was a marked increase in the rate of flow.

The *chyle* injected contained but little cholesterol; analyses have not been reported because accurate determination has been impossible due to color changes which interfered with the colorimetric procedure. However, so little cholesterol was contained in the chyle that on intravenous injection no increase in blood cholesterol was noted immediately afterward. Correspondingly, no rise in the lymph cholesterol was obtained. The *fat emulsion*, however, did contain an appreciable amount of cholesterol (206 to 425 mgm. per cent); although its injection raised the level of blood cholesterol, no effect was seen on the cholesterol content of the lymph. Apparently cholesterol in the form injected has great difficulty in finding its way through the capillary wall. Although during the period of 4 hours following an injection of fat the fatty acid content of the blood gradually fell, the level of blood cholesterol remained quite constant.

Rony, Mortimer and Ivy (1932) found that in dogs even the fasting thoracic duct lymph contained from 0.249 to 1.030 per cent fatty acids. Under such conditions the thoracic duct lymph uniformly contained more fat than the blood examined simultaneously. They argue from this that the fat present in the lymph comes chiefly from the reserve fat of the depots and tissues. They do not believe that the blood fat enters the lymphatics directly after leaving the blood. This conclusion is based on an experiment in which "two dogs were fed each $\frac{1}{2}$ pint of cream and four egg yolks. Four hours later the cervical lymph duct was cannulated and lymph collected for 1 hour. Immediately following this, the thoracic duct was cannulated. The clear cervical lymph contained 200 mgm. and 210 mgm. of fat per 100 cc., respectively, while the milky thoracic duct chyle contained 1580 mgm. and 4500 mgm. of fat per 100 cc." Unfortunately, no control values before the giving of the fat meal were obtained and no blood values were reported.

Man and Peters (1933) determined the protein, fatty acid, lipoid phosphorus, and cholesterol content of the blood serum of five normal persons before and after they had stood still for 30 minutes. They found that in every instance the cholesterol increased in approximately the same proportion as the serum protein. In 5 of the 7 experiments, the lipoid phosphorus and the fatty acids likewise increased in about the same proportion as the serum protein. They further determined simultaneously the protein and

lipoid content of the blood serum and various transudates. They found in general that no correlation existed between the concentrations of cholesterol, fatty acids or lipoid phosphorus in the sera and in the transudates studied. In most instances, however, the content of these substances and of protein in the transudates was proportional. Man and Peters conclude that the blood capillary wall is normally impermeable to cholesterol, phosphatids and compounds of fatty acids.

Our results are entirely in accord with those of Man and Peters (1933) in so far as cholesterol is concerned. As regards compounds of fatty acids, however, there does seem to be a real tendency for the direct passage of small amounts from the blood stream into the tissues. The increases seen in the total lipid content of the cervical lymph are small but definite. We cannot prove that the increase in fatty acids does not represent mobilization of fat from the tissues, but on the other hand there is no direct information to support such a contention.

SUMMARY

1. The peripheral (cervical) lymph of normal fasting dogs contained on the average per 100 cc. of lymph the following: cholesterol, 56 mgm.; fatty acid, 239 mgm.; and total lipid, 305 mgm. These values are respectively 41 per cent, 54 per cent, and 52 per cent of those for cholesterol, fatty acid, and total lipid in blood plasma from the same dogs. Blood and lymph samples were obtained as nearly simultaneously as possible.

2. Following the intravenous injection of fat-laden chyle or of a fat emulsion, there usually occurred within 1 to 4 hours a slight rise in the total lipid content of the cervical lymph. Under such conditions no significant change occurred in the cholesterol content of the lymph despite the production at times of a mild hypercholesterinemia.

3. These experiments suggest that the degree of permeability of the blood capillary wall to lipoid substances other than cholesterol is slight but definite and is greater than that to cholesterol itself.

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HYPOGLYCEMIA HYPOPHYSIOPRIVA

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In a series of hypophysectomized puppies the observation was made that the animals, though making an apparently perfect recovery for twenty-four hours, then went into that peculiar state which was called "cachexia hypophyseopriva" by Cushing (1). This state so strongly suggested what is now known as hypoglycemic shock that the blood of the animals was examined, and found to be very low in carbohydrates. Administration of sugar resuscitated them.

The preparation of the puppies was undertaken at the request of Dr. H. M. Evans in January 1933, and a preliminary report (2) of these results was made before the New York Surgical Society on March 22, 1933. Meanwhile Keller and D'Amour (3) have corroborated the blood sugar studies in dogs, and White (4) has made a similar observation in rabbits.

Cushing used the term "cachexia hypophyseopriva" in 1909, " . . . . in imitation of Kocher's designation for the symptoms provoked by a total thyroidectomy." Later the state was described in detail by Crowe, Cushing and Homans (5), who characterized it by a loss of the usual interest in surroundings, irritability, unsteadiness of gait, arching of the back, lethargy, fine muscular twitchings, hypersalivation, convulsions, hypothermia, coma, and death. The symptoms occur spontaneously after about three days in adult dogs, but after twenty-four hours in very young puppies. This usual course after hypophysectomy led to the feeling that life was incompatible without the pituitary body.

Because of its anatomical seclusion the hypophysis repeatedly resisted successful removal, and a variety of operations was developed. Fatalities were frequently attributed to trauma which the hypothalamus suffered, so that as late as 1925 Dandy and Reichert (6) proposed refinements for the Paulesco subtemporal procedure, since they subscribed heavily to the traumatic theory in explanation of cachexia hypophysiopriva. They used the "hanging brain" position at operation, and Weed's method of reducing intracranial volume with hypertonic sodium chloride. They felt that the cachexia subsequent upon hypophysectomy was due to faulty technique, for they did not recognize the humoral imbalance now demon-

strable by the blood chemical studies which were not at the disposal of workers twenty-five years ago. Microscopical studies (7) of the hypothalamus have disclosed no evidence favourable to the traumatic theory of cachexia in the present series; but there was an associated hypoglycemia: hence the use of the term *hypoglycaemia hypophysiopriva.*

The present observation was made while nursing hypophysectomized puppies of five to ten weeks of age, preparatory to injection experiments. Puppies of this age were used, in spite of their fragility, because the arrest of growth served as a criterion of complete hypophysectomy. The difficulties of handling them were more than compensated for by their susceptibility to acute cachexia hypophysiopriva, whereby the train of symptoms was so quickly and repeatedly presented that the carbohydrate deficiency could not be overlooked.

The operative procedures were carried out under sodium amytal anesthesia administered intraperitoneally, 0.5 cc. of 10 per cent solution per kilogram of body weight. This anesthetic is reported to cause no alteration in the blood sugar (8). The right subtemporal approach to the gland was used in all instances, and, with the added help of hypertonic sodium chloride given intravenously, as recommended by Dandy and Reichert, exposure of the hypophysis was excellent even without resort to the manoeuvre of the "hanging brain." The gland was carefully dissected from the shallow canine sella and removed with a sucker. The post-operative care of these animals was most important, and frequently more than trying to the ingenuity and patience of the operator who became nurse immediately when the closure was completed.

After recovery from anesthesia the puppies were normally playful, but within twenty-four hours they were hyperactive, irritable, and had lost appetite and interest in the surroundings; the back arched, the tail was carried between the legs, fright occurred without adequate cause, fine muscular twitchings set in, also lethargy, diarrhoea, salivation, vomiting, hypothermia, hypotension, convulsions, coma and death. These difficulties occurred even after the most meticulous operative attention. Post-mortem studies revealed nothing to explain such fatalities.

After observing the same picture day after day, its resemblance to the hypoglycemic crises of man came to mind, and glucose was given intravenously to a comatose convulsive puppy. There was an almost immediate and startling change, the animal recovering from coma and becoming lively as a normal puppy. The cachectic state soon recurred, but was relieved again by glucose. These alternating pictures of coma and recovery followed the depletion and replenishment of the carbohydrate storehouse of the animal. The intravenous method of glucose administration was not alone effective, for subcutaneous and intraperitoneal injections, as well as gastric feedings, brought results though in a much slower and less dramatic manner.

The puppies had been given no food for fifteen hours or more before operation, nor any post-operatively for another thirty hours. By that time they had no appetite, or would not eat, the symptoms already having been established. Efforts made to raise the available glucose both pre- and post-operatively in the subsequent undertakings checked the development of the usual cachexia, but this necessitated forced feedings by stomach tube, in some instances for a fortnight, until the animals began to eat voluntarily.

Blood-sugar readings on fasting control and hypophysectomized puppies were striking. The fasting anesthetized (sodium amytal) control puppies

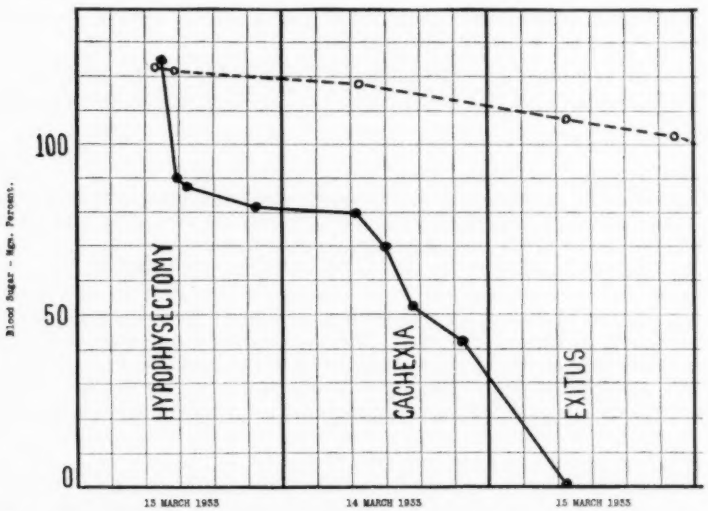

Fig. 1. Broken line: fasting control puppy. Solid line: hypophysectomized puppy

manifested only the slightest depreciation in blood-sugar content over periods of three days, in contradistinction to the fasting hypophysectomized puppies. These latter maintained a good blood-sugar level for about twenty-four hours, but there was then a marked drop which reached zero within thirty-six to forty hours when the animals died. The signs of cachexia were to be noted at the 50 mgm. level (fig. 1).

Other observations than the previously mentioned carbohydrate changes were of interest in these hypophysectomized puppies. The usual hypoplasias of the glands of internal secretion, except the pancreas, were observed in the dogs maintained over a long time. The pancreas was always grossly altered, having a swollen appearance with mulberry color, but no constantly associated histological changes were to be seen. The adrenal

cortex showed occasional degeneration upon microscopical study, and about the central vein of the liver there were cellular changes of vacuolar nature, but without alteration of the nuclei. This was not a true necrosis, although it resembled such under low magnification, but may have been due to withdrawal of glycogen.

In no instance was the gastro-intestinal tract ulcerated. The hypothalamic nuclei were unaffected when studied histologically. This excludes the traumatic hypothesis to explain the "cachexia." Further negative observations were the absence of obesity, polydipsia, and polyuria in all of these hypophysectomized dogs whether the survival period was one day or one year. At no time was sugar found in the urines which were obtained and examined immediately after operation. Blood sugars have been subnormal and the glucose tolerance high.

Protocols typical of the observations reported above are detailed. An early experience with the usual symptoms of cachexia hypophysiopriva is the following:

OBSERVATION 6. Male puppy: aged 7 weeks; weight 3 kgm.

30 January 1933. Operation: complete hypophysectomy by the right subtemporal route under sodium amytal anesthesia. Pituitary body removed at 11 a.m. Recovery from anesthesia at 8:30 p.m. in apparently normal condition.

31 January 1933. Behavior like that of an average puppy until 1:30 p.m. Then a personality change was evidenced by irritability, restlessness, fear, unexplainable howling, carrying of the tail between the legs, arching of the back, and running to hide, ostrich like, in corners of the cage. At 4 p.m. the first food presented in two days was refused. Soon there was weakness of the hind legs, then of the entire body, so that the animal staggered and finally collapsed having generalized fine tremors, kicking the legs aimlessly, salivating excessively, and passing loose faeces. Saline hypodermoclysis of 100 cc. was given without improvement in the condition. Progression of the cachexia to coma and exitus at 11 p.m.

Post-mortem examination revealed no explanation for the fatality, there being no hematoma, no evidence of infection, nor gross damage to the cerebrum. Histological examination did not demonstrate either hypophysial tissue or injury to the hypothalamic nuclei.

The effect of supplying glucose to a puppy in the advanced state of cachexia hypophysiopriva is detailed in:

OBSERVATION 10. Female puppy: aged 5 weeks; weight 2.3 kgm.

6 February 1933. Operation: under sodium amytal anesthesia the entire hypophysis was removed by the right subtemporal route at 11:30 a.m. Recovery from anesthesia at 7:15 p.m.

7 February 1933. Condition excellent; normally playful. Took water and milk. At 5 p.m. refused solid food, was irritable, moved about aimlessly, shivered, and was unsteady.

8 February 1933. Found moribund at 8:30 a.m., untidy, salivating excessively, breathing slowly but noisily, the four extremities almost constantly in motion as though running as it lay comatose. Venepuncture was done with great difficulty

because of collapse of the veins, the result of generalized hypotension. Blood sugar 24 mgm. per cent. The introduction of 10 cc. of 50 per cent glucose intravenously was followed by an almost immediate and very startling response, for the animal promptly became conscious, and within two minutes was walking about and playing. Coma recurred after two hours, and administration of glucose intravenously again resuscitated the animal. It continued in a lively state for another hour, but was dead when next observed at 12:45 p.m.

Post-mortem examination again gave no tangible explanation for the fatality, nor was any change to be seen in the supra-optic or paraventricular nuclei to be linked with the carbohydrate disturbance.

The result of high carbohydrate maintenance before and after operation is demonstrated by:

Observation 15. Female puppy: aged 9 weeks; weight 4 kgm.

19 February 1933. Given high carbohydrate diet.

20 February 1933. Pre-operative milk and sugar feeding. *Operation:* complete hypophysectomy under sodium amytal anesthesia by the right subtemporal approach at 11 a.m. Hypodermoclysis of 150 cc. of 5 per cent glucose in Ringer's solution at end of operation. Recovery from anesthesia at 10 p.m. Given milk and sugar to drink.

21 February 1933. Condition good. Slight ptosis of right upper eyelid. Right pupil dilated. Played normally, took fluids, and ate meat.

22 February 1933. Took water but refused both liquid and solid food. Given repeated forced feedings of lactose eggnog.

23 February 1933. Active and playful, but took no nourishment; forced feedings continued.

24 February 1933. Generally in good condition. Pupils equal. Ptosis no longer present. Still required forced feedings.

26 February 1933. More playful. Eating voluntarily.

1 March 1933. Appetite and condition excellent. Weight 4.1 kgm.

11 March 1933. Eating less than litter mates, but was as active as they. Weight 3.8 kgm.

12 April 1933. Generally the same. Coat in poor condition. Weight 4 kgm.

31 May 1933. Condition good. Quite active and playful. Weight 4.4 kgm. Weights of control litter mates: male, 12 kgm.; female, 11.5 kgm. Sacrificed for histological study. Pituitary body entirely absent. Hypothalamic nuclei normal in Nissl preparations. The genitalia and glands of internal secretion were hypoplastic. The pancreas appeared swollen and was of mulberry color; but there was no demonstrable histological alteration.

The gathering of experimental evidence in the field of carbohydrate-pituitary relationships was begun in 1908 by Borchardt (9), who observed glycosuria in dogs and in rabbits after subcutaneous injections of pituitary gland extract. Later followed Burn's (10) work wherein posterior lobe extract was found to cause a rise in blood sugar, and the curve obtained was the antithesis of that which followed insulin injection. It was also noted that if insulin and posterior lobe extract were injected at the same time there was no alteration of the blood sugar.

Evans (11) and his group, while carrying out studies on the growth principle by injections, noted that their gigantic animals were not well.

The experiments had gone on for eight months. Sugar was found in the urines, and the blood sugar was elevated to well over 200 mgm. After discontinuing injections the diabetic-like state disappeared in two to three weeks. More recently Houssay (12) has produced a diabetic likeness in dogs in a few days by using massive injections of crude anterior lobe extract.

In contradistinction are the detailed ablation experiments of Cushing and his co-workers (5) (13) (14) who, while trying to determine if the pituitary body was essential to life, were drawn into the study of carbohydrate metabolism after total and partial hypophysectomies. They had expected to find glycosuria after extirpation of the gland, but the urines did not reduce Fehling's solution. By feeding high carbohydrate diets they determined that a state of increased tolerance to sugars existed, and attributed this to deprivation of the posterior lobe. They also noted that injections of posterior lobe extract lowered the previously developed high tolerance to carbohydrates.

It is of special interest that these workers had touched upon the pancreatic-pituitary relationship, and in a series of dogs in which the pancreas had been almost completely removed there followed the "usual spontaneous glycosuria" (13). After an interval these animals were hypophysectomized and a carbohydrate tolerance "practically twice the normal" was observed. The tolerance was then reduced by the injections of posterior lobe extract, and the animals lost weight while so treated. Houssay and others have more recently done combined hypophysectomy and pancreatectomy, and the sequellae which usually follow the removal of either pancreas or pituitary body alone did not occur.

The clinical observations on carbohydrate metabolism in relation to the pituitary body were begun in 1884 by Loeb (15). He noted "mellituria" in a patient found to have a so-called pituitary sarcoma. The observation was soon verified by Pierre Marie (16) in some acromegalics. This malady is now known to have associated glycosuria in 25 to 40 per cent of instances (17).

So also in basophilic hyperpituitarism (18) (19) both glycosuria and hyperglycemia have been observed in about 50 per cent of the studied cases. In both the acidophilic and basophilic hyperpituitary states the sugar tolerance is low. And singularly, the "diabetes" associated with these maladies is especially resistant to the ordinary management for diabetes mellitus.

The pancreatic pathology in acromegaly is not marked, and demonstrable lesions are as inconspicuous as they are in diabetes mellitus. However, in diabetes mellitus the hypophysis has been found to contain adenomatous masses of eosinophile cells and excess of colloid which have been interpreted as evidence of hyperactivity (20). On the other hand, after almost total pancreatic extirpation in cats degenerative changes have been noted in the pituitary body (21).

Other than those acromegalics who have glycosuria, some with a higher kidney threshold are unable properly to manage glucose as demonstrated by the usual high blood sugar tolerance curves. More interesting are the curves following the extirpation of these patients' acidophilic adenomata, for then the blood sugar levels and glucose tolerance curves are distinctly lower (17). On the other hand, patients with chromophobe adenomata presenting the picture of hypopituitarism almost never manifest glycosuria, but rather hypoglycemia with high tolerance for sugar.

The exact site of origin of a pancreatic balancing principle of the pituitary body is not definite. The clinical manifestations of acromegaly and pituitary basophilism with lowered tolerance for carbohydrates which has been returned to normal after operation upon, or irradiation of, the associated anterior lobe adenomata, direct our attention to the secretory part of the pituitary body. Increased tolerance to glucose has been observed after removal of either anterior or posterior lobe alone, although it must be added that the intimate anatomical relationship of the anterior lobe's pars tuberalis to the posterior lobe precludes exact extirpation of either one or the other division.

Injection of either anterior or posterior lobe principles has been followed by an elevation of the blood sugar. These data taken together are confusing. It does, however, seem more likely that the anterior lobe is responsible, for it may actually be the only source of pituitary secretion when it is realized that there are no anatomically recognizable secretory elements in the pars nervosa. But this conclusion cannot be drawn directly without detailed study of the neurohypophysial mechanism as a whole, of which our knowledge is yet so limited. It may be that the intracranial carbohydrate regulatory influence is composed of humoral and neural links, powerful when coupled, though individually much less effective.

SUMMARY

The state of cachexia hypophysiopriva has long been known to follow hypophysectomy, and has been attributed by some observers to operative trauma *per se*. In a series of hypophysectomized puppies which succumbed with this syndrome, no lesions could be demonstrated in the hypothalamus to bear out the traumatic theory, but hypoglycemia was encountered. Administration of glucose raised the blood sugar and resuscitated animals in the advanced state of this cachexia, so hypoglycemia hypophysiopriva is offered as an explanation for the previously mysterious fatalities following hypophysectomy.

In fasting puppies the physical signs of hypoglycemia appear within twenty-four to thirty-six hours after hypophysectomy, at a blood sugar level of 50 mgm. (Benedict-Folin-Wu); following this the blood sugar curve falls rapidly to zero.

Repeated administration of carbohydrates has prevented the usual cachectic course after hypophysectomy. The glucose combustion curves of hypophysectomized dogs, being low, indicate a high tolerance.

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SPIRAL PROPULSION OF A BOLUS IN THE INTESTINE

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The longitudinal and circular muscles of the small intestine according to Carey (1) are arranged in a spiral manner and a bolus moves in a counter-clockwise spiral path (as viewed from the cephalic end) in an excised intestinal loop. In five dogs given apomorphine preceding an abdominal operation we observed an apparent spiral contraction of the small intestine and were thus stimulated to investigate the physiological significance of this phenomenon, particularly since the implications of Carey's report have not been generally accepted.

A variety of experimental methods were employed in this investigation.

EXPERIMENTAL. Thiry-Vella loops were prepared from the second thirty-five centimeters of the jejunum in six dogs. The loops were placed transversely to the body axis and during the week which elapsed between operation and initiation of experimentation the animals were trained to lie quietly on comfortable mattresses. The operation was performed with surgical technique under anesthesia.

Cotton boli of two sizes (1 by 2 cm. and 1 by 4.5 cm.) were prepared and two long threads of different colors attached by one end to opposite sides of each bolus and by the other end to opposite sides of a piece of flexible wire. After soaking a bolus in warm water it was inserted into the proximal end of the intestinal loop and permitted to move forward; the exposed portions of thread being kept separated. Spontaneous expulsion of the bolus from the anal end of the loop would occur, but during this process rotation of the bolus was noted. This rotation did not appear to be an artifact related to the passage through the fistular opening for it always occurred in the counter-clockwise direction; but we prevented such a possible error by removing the bolus with a forceps upon its arrival at the distal aperture. Holding the bolus and the flexible wire so as to prevent rotation, the wire was drawn through the intestinal loop. Examination of the threads gave accurate information regarding the extent and direction of rotation undergone by the bolus in passing through the intestinal segment.

As noted in table 1, rotation of the bolus did not invariably occur but when observed, it was always in the counter-clockwise direction. A portion of the "failures to observe rotation" especially in the short loops arose from the fact that we did not record rotation unless it exceeded 45°. Rotation of the short bolus was noted more uniformly than of the long bolus. The former rotated about 180° in the short (15 cm.) loops, 270° to 360° in the 23 cm. loop and quite uniformly 360° in the 35 cm. loop. Rotation of the long bolus was usually 90° to 180° in 15 to 23 cm. loops. The extent of rotation did not vary appreciably in the individual animal but exhibited moderate variations between different animals. Observations on rotation of the bolus were made only when propulsion rate was normal (2) (3).

TABLE 1

Showing the frequency at which rotation of a bolus occurred on passage through a Thiry-Vella fistula of the jejunum

DOG	POST-OP. LENGTH OF FISTULA	TRIALS WITH SINGLE SHORT BOLUS		TRIALS WITH SINGLE LONG BOLUS		TRIALS WITH DOUBLE BOLUS	
		No rotation	Rotation	No rotation	Rotation	No rotation	Rotation
	cm.						
1	15	6	24				
2	15	46	131	7	4	0	10
3	23	0	40	16	32	0	10
4	23	0	40	4	46	0	10
5	23	0	50	24	36	0	10
6	35			0	11	0	10
Total.....		52	285	51	129	0	50

Rotation was always to the left (counter-clockwise) when viewing the loop from the cephalic end.

In these tests rotation was not recorded unless it exceeded 45°.

A complete rotation of the bolus sometimes occurred in a 15 cm. loop but not invariably in a 23 cm. loop. The bolus was rotated 360° in the 35 cm. loop in eight of the eleven trials on different days. Apparently from 23 to 35 cm. of jejunum must be traversed by the bolus as a rule before it is rotated 360°.

Since the cotton bolus is inferior in certain respects to a sponge rubber bolus (3) for the study of intestinal motility we repeated and extended the above investigations by similar methods in 3238 observations using the 0.8 by 2.5 cm. rubber bolus. Thiry-Vella loops 9 cm. long within the abdominal cavity made from the upper jejunum or lower ileum in nine dogs were employed. The results were sufficiently similar to those obtained with the cotton bolus to permit the following summarization. The ob-

served rotation of the rubber bolus was invariably in the counter-clockwise direction. The extremes of rotation varied from 0 to 180° but the usual result was near 60°. The rotation per centimeter was less than with the cotton bolus apparently due to the employment of shorter loops in which the observational error was greater. On the other hand "failures to observe rotation" were much less frequent with rubber boli because in these tests a minimal rotation limitation of 45° was not imposed. Rotation in jejunal and ileal loops appeared the same, it was constant for periods of months during which the loops were used daily to prevent disuse "atrophy" and thus was the same in freshly prepared or in old Thiry-Vella loops. The degree or constancy of bolus rotation was not modified by alimentation, by introducing 3 cc. of mineral oil into the loop or by the administration of moderate doses of morphine, papaverine, atropine, pilocarpine, pituitrin, pitocin, pitressin or glucose. Certain of these drugs modified propulsion rate markedly (4) but since they did not alter rotation, the latter activity appeared to be independent of propulsive activity under such condition.

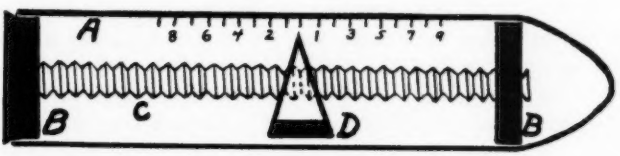

Fig. 1. Gyrometer. Glass tube *A*, with rubber blocks *B*, supporting screw *C*. Rotation of gyrometer causes indicator *D* to move along the screw; extent and direction of rotation indicated on the scale.

We devised a "gyrometer" enclosed within a glass tube 1 by 4.5 cm. When this device was inserted within the intestinal lumen, it recorded the number and direction of revolutions of the glass tube. It possessed the minor objection of only recording accurately when within 45° of the horizontal. The gyrometer was introduced into the intestine through jejunal fistulae in five otherwise normal dogs. When passed in the feces it recorded an average of 4 counter-clockwise rotations. We were unable to devise a more efficient "gyrometer." (Fig. 1.)

To ascertain the effect of passage of a bolus on the rotation of a second bolus closely following, two short cotton boli were fastened together by two threads three inches long attached to the sides of the boli. Two pendent threads were attached to the second bolus as described above. When these tandem boli were allowed to pass through a Thiry-Vella loop the usual twisting of the pendent threads was noted and since the threads connecting the boli were not twisted, counter-clockwise rotation of each bolus must have occurred in the same degree.

A thin lead plate notched differently on each edge placed within a short bolus was observed fluoroscopically to rotate while passing spontaneously through a Thiry-Vella loop.

The investigations on the Thiry-Vella loop were amplified by two experiments on the normal intestine. Under anesthesia a small bolus with pendent threads was inserted into the jejunal lumen, the fistular opening was fastened to the abdominal wall, the anesthetic withdrawn and the bolus allowed to move downwards eight feet. The animal was then sacrificed (ether intravenously), the intestines fixed with formalin and opened along the antimesenteric border, the threads being fixed with wax as exposed every 15 cm. In one dog the bolus had rotated in the counter-clockwise direction 12 turns, in the other dog 6 turns. Actually the bolus was propelled along the intestine for about 9 feet for in each animal an intussusception of the small intestine had occurred, apparently due to a puckering of the intestine along the thread. Thus the boli in these experiments made an average of one rotation in passing 34 cm. In both dogs the bolus had passed into the cecum.

Discussion. With the exception of the x-ray studies, the methods used for investigating rotational activity of the intestine all have the objection that they record the *algebraic sum* of the bolus or gyrometer rotations. Indications that clockwise rotation ever occurs in the intestine were not encountered in this investigation although they were sought for continually and especially in the x-ray studies. The results therefore appear to indicate accurately the type and to a lesser degree the extent of rotational activity of the intestine. If rotation of the bolus is a consequence of the intestinal structure, rotation as we noted should not be modified by alimentation, drugs, etc. However, on this basis it is difficult to explain the wide range of results obtained under apparently constant conditions but it may indicate that the observational methods are at fault. Our results strongly indicate that the propulsive force of the intestine is so exerted as to force intestinal contents in general in a spiral path. This type of movement, however, may be more marked with solid or semi-solid material than with liquids.

SUMMARY

Spontaneous propulsion of a cotton or rubber bolus or a gyrometer through a Thiry-Vella loop of the intact intestine was shown to occur with a counter-clockwise rotation, confirming under a variety of more physiological conditions the report of Carey. A similar conclusion resulted from x-ray studies. The rotational propulsion was apparently the same in the jejunum and ileum and in these experiments was not altered by alimentation or by a variety of drugs.

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A STUDY OF THE BLOOD VOLUME OF ADRENALECTOMIZED DOGS[1]

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Few investigators have studied the syndrome of adrenal insufficiency without commenting on the hemoconcentration which becomes so apparent as symptoms progress. The fact that blood concentration occurs in insufficiency has long been known, but for the most part, the phenomenon has been regarded as but one of many changes which appear, and it has generally been considered as a terminal event, and not as a primary and initiating factor in the syndrome.

The following references offer data or comments on various aspects of the question (Gradinescu, 1913; Donath, 1914; Kellaway and Cowell, 1923; Rogoff and Stewart, 1926; Banting and Gairns, 1926; Lucas, 1926; Swingle, 1926; Swingle and Eisenmann, 1926; Hartman, MacArthur, Gunn, Hartman and MacDonald, 1927; Viale and Bruno, 1927; Swingle and Wenner, 1928; Wyman and Walker, 1929; Harrop, Pfiffner, Weinstein and Swingle, 1932; Swingle, Pfiffner, Vars, Bott and Parkins, 1933; Harrop, Weinstein, Soffer and Trescher, 1933; Pfiffner, Swingle and Vars, 1934; Zwemer and Sullivan, 1934, and others).

Reference to the various methods employed for determining blood pressure, blood urea nitrogen and hemoglobin are given in earlier papers of this series of adrenal studies (Swingle, Pfiffner, Vars and Parkins, 1934; Parkins, 1934). The type of experimental animal used has also been adequately described in the same references, and in a preliminary paper in Science, 1933.

The essential data, in so far as the writers' observations on blood concentration in adrenal insufficiency are concerned, are presented in figure 1 and table 1. It is apparent that the hemoconcentration of adrenal insufficiency is due to changes in plasma volume. Since it seemed that it is due to alterations of volume of circulating fluid (and associated electrolyte changes, Loeb et al., 1933; Harrop et al., 1933) that the syndrome of adrenal insufficiency takes its origin, a detailed study of the blood volume by both dye and Welcker methods was undertaken.

[1] These investigations have been aided by a grant from the Josiah Macy Jr. Foundation, New York.

The methods for blood and plasma volume determination now in current use appear to be none too satisfactory even under the best conditions, and especially when they are applied to adrenal insufficiency. However, they seem to be the best available, and give results which at any rate are an approximation of the actual condition existing in this syndrome.

A. *Blood volume of the adrenalectomized dog—dye method.* Several groups of workers have studied the plasma volume of adrenalectomized

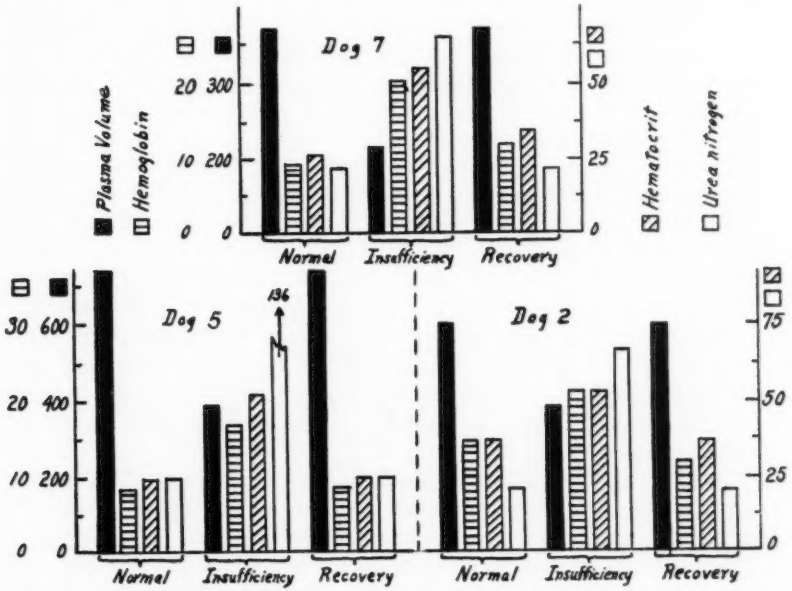

Fig. 1. Changes in plasma volume, cells by hematocrit, hemoglobin and blood urea nitrogen of three adrenalectomized dogs followed through control, insufficiency and recovery periods. Dog 5 had been adrenalectomized five months, dog 7 ten months, and dog 2 twelve months previously, and maintained in normal condition by adequate doses of cortical hormone. The urea nitrogen of dog 5 at the height of insufficiency was 136 mgm. per cent.

animals using the dye technique. Kellaway and Cowell (1923) used one adrenalectomized cat but the result was so unsatisfactory they abandoned the method with the comment "It is obvious that the method (vital red) is not applicable to this problem as it seems likely that in adrenal insufficiency the permeability of the capillary endothelium has altered so that the dye is removed from the circulation faster than in the normal animal."

Rowntree and Brown (1925) reported that the blood volume of patients with Addison's disease was not diminished even in severe cases. How-

ever, in a later report (1929) they state that there is a relationship between the blood volume and the clinical status of the patient. The patients in shock and collapse showed the lowest volumes of blood; improvement was followed by an increase both in blood volume and in plasma volume.

Wyman and tum Suden (1930) studied the blood volume of adrenalectomized rats and demonstrated that in these animals there is a reduction in the total blood volume, due chiefly to a reduction in the plasma volume during the final stages of insufficiency when severe symptoms appear.

Kendall, Mason, McKenzie and Myers (1933) working on adrenalectomized dogs report that the blood volumes of the animals were low at the time of death. They considered this, however, as only one of many changes which develop and did not regard the volume changes as of primary importance.

Harrop, Weinstein, Soffer and Trescher (1933) calculated the plasma volume of adrenalectomized dogs in insufficiency from the hematocrit values, and state that the loss of plasma volume as judged from the volume of packed erythrocytes may be from 10 to 35 per cent in severe insufficiency.

Britton and Silvette (1933) report that the blood volume of adrenalectomized cats exhibiting symptoms, commonly (but not in all cases) was reduced. In 12 experiments there was an average decrease of 20 per cent. Decreases of 30 per cent were observed in a few cases. These authors assert that several animals dying of insufficiency had blood volumes within normal limits. They did not regard the changes in blood volume as of primary significance.

The present writers in a preliminary communication (1933) and in recent papers (1934) have presented evidence on the importance of the blood and plasma volume in adrenal insufficiency.

The Congo red method as outlined by Rowntree and Brown (1929) was used. The adequacy of this method for determining the plasma volume of normal dogs had been criticized by various workers. In our hands, the accuracy of the method on duplicate determinations in the adrenalectomized dog maintained in normal health by adequate injections of cortical hormone, was approximately 5 per cent. Some of the difficulties and probable errors attendant upon use of the dye method in adrenal insufficiency will be pointed out later following presentation of the actual experimental findings.

Following withdrawal of cortical hormone, the plasma volume of the adrenalectomized dog declines simultaneously with a rise in hemoglobin, cells by hematocrit and blood urea nitrogen. After injection of hormone into the dogs verging on prostration from insufficiency, the plasma volume slowly rises, and the hemoconcentration disappears. Clinical improvement parallels the increase in plasma volume and arterial pressure, and the decrease in blood concentration (fig. 1).

It will be observed in figure 2 that five of the animals were terminal cases, i.e., they were allowed to die and no extract was given. The remainder of the cases were returned to normal health by hormone injections. The plasma volume of the fifteen animals (sixteen experiments) in insufficiency decreased from 13 to 56 per cent, with an average decrease of 33 per cent.

Terminal plasma volume figures obtained on the series of five dogs (nos. 1, 3, 4, 9 and 13) ranged from 20 to 46 per cent with an average decrease of 36 per cent. Five animals (nos. 6, 8, 10, 11 and 12) allowed to come into

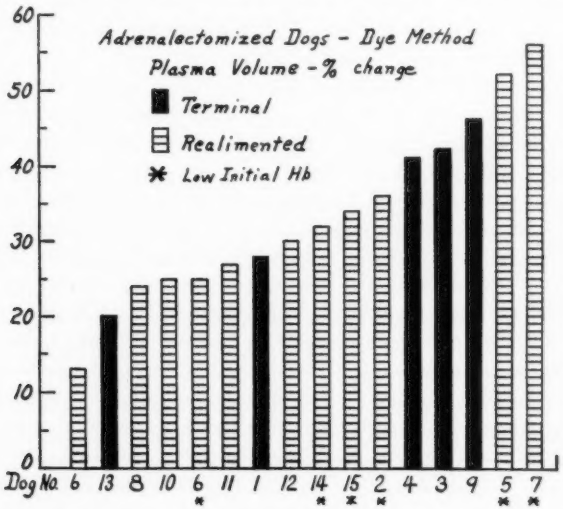

Fig. 2. The plasma volumes of fifteen adrenalectomized dogs (sixteen experiments since dog 6 was used twice) during insufficiency, expressed in per cent of change from the normal. The plasma volumes of all animals were determined during control period, at intermediate stages and again at or near the height of insufficiency or just before hormone administration.

insufficiency, showed an average decrease of 23 per cent with a range of 13 to 30 per cent before being restored to normal condition.

In general, the largest plasma volume changes were observed in the series of six dogs that had been adrenalectomized 4 to 12 months (dogs 2, 5, 6, 7 and 14 are examples) previously and which were brought into insufficiency by gradual reduction of hormone administration over periods of 4 to 8 weeks. The average plasma volume decrease in this group was 39 per cent with a range of 25 to 56 per cent. It should be noted that in this group of six animals, the average hematocrit was only 31 with a range of 24 to 42 as compared to an average hematocrit of 41 with a range of 29 to 46 in the remainder of the series. The largest deviations from the normal

in plasma volume, 52 to 56 per cent respectively, occurred in two dogs which had hematocrit reading of 24 and 26 (dogs 5 and 7).[2]

The hematocrit (including both groups having a normal and a low initial hemoglobin) increased in the series of 15 animals during insufficiency by an average of 33 per cent. In the five terminal cases with normal initial hematocrit, the increase averaged 24 per cent, in five animals in insufficiency 13 per cent, while in the group of six dogs that had a low hematocrit at the beginning of the experiment the increase averaged 57 per cent. Two animals having initial hematocrit reading of 24 and 26 respectively showed increases of 111 and 112 per cent. Complete data on one animal (no. 9) are given in table 1.

Critical comment on the use of the dye method in adrenal insufficiency. The wide variation with regard to the degree of change in the plasma volume of the 15 dogs (16 experiments) i.e., from 13 to 56 per cent decline from the normal, calls for comment. It is probable that the degree of insufficiency at the time the volumes were determined varied in the different cases. However, it is conservative to state that the dogs were exhibiting definite and unmistakable symptoms of insufficiency at the time the volumes were determined. The cause of the wide variation in plasma volume is of interest, and raises the question of the applicability of the dye method to conditions where circulatory failure is a prominent symptom.

No stress has been laid on the whole blood volumes where the dye method has been employed, because of the criticisms of various investigators (e.g., Smith, Arnold and Whipple, 1921, and others) against the assumption that the hematocrit readings obtained from jugular blood are in any way representative of conditions existing throughout the vascular bed. In adrenal insufficiency the jugular hematocrit values are very probably not representative, but they were the best that could be obtained.

It seems that in some of the animals during insufficiency, the intensity of the vasoconstriction is more marked than in others with the result that large areas of the circulation are practically shut down thereby blocking great masses of the corpuscles in the liver, spleen, pancreas and gastrointestinal region. Marked vascular stagnation and congestion occur, the blood viscosity is high and the rate of flow greatly decreased. Under such conditions what little plasma there is in circulation is perhaps freer than the formed elements to drain sluggishly to the larger vessels (e.g., jugular) thereby giving hematocrit readings which may not in any way reflect the true condition of stagnation and collapse existing in the vascular system. Shunting of blood from the less essential peripheral and muscular regions to the vital central organs and brain must occur in order to maintain life.

[2] The Hb of bilaterally adrenalectomized dogs is generally, if not invariably, considerably lower than normal. The experimental data indicate that the lowered Hb is due to adrenal removal itself and not to dietary or other disturbing factors.

TABLE 1

Blood and plasma volume (dye method) water balance and correlated blood and urine changes in adrenalectomized dog 9

DATE	WEIGHT	PULSE	BLOOD VOLUME	PLASMA VOLUME	HEMATOCRIT (5 CC.)	HEMOGLOBIN	BLOOD SOLIDS	SERUM SOLIDS	BLOOD UREA NITROGEN	BLOOD NON-PROTEIN NITROGEN	BLOOD SUGAR*	WATER	FOOD	TOTAL WATER INTAKE	VOLUME URINE	SPECIFIC GRAVITY URINE	REMARKS
1932	*kgm.*		*cc.*	*cc*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*grams*	*grams*	*grams*	*cc.*		
March 23	15.76	42	1165	745	36.1	12.8	17.3	6.4	22	39	62	438	837	959	350		Normal condition
24	15.33											369	910	935	525	1.030	Normal condition
25	15.33	40				12.2	17.1	6.4			66	485	880	1040	630	1.027	Normal condition
26	15.65											464	904	1031	670	1.024	Normal condition
27	15.76											504	907	1076	605	1.026	Normal condition
28	15.88	40	1159	740	36.0	11.5	16.8	6.3	24	41	71	566	907	1135	725	1.024	Normal condition
29	15.99											585	907	1152	735	1.025	Extract discontinued
30	15.88	64				12.7	18.1	6.9	20	35	77	411	907	983	755	1.026	
31	15.20	64	1010	623	38.2	12.7	18.4	7.2	30	46	58	364	538	683	400	1.030	Animal vomited food
April 1	15.08	64				14.1	19.4	7.3	45	62	58	320	9	285	165	1.040	
2	14.85	72	905	499	44.7	14.3	19.7	7.3	36	52	58	0	0	0	193	1.040	
3	14.51	96				14.3	20.3	7.4	53	75	58	183	8	141	165	1.036	Symptoms—animal weak
4	14.10	108	866	398	54.2	17.8	21.5	7.7	91	144	58	0	12	0	65+	1.021	Marked symptoms—animal found dead next morning

* Somogyi copper filtrate.

The conditions enumerated undoubtedly vary in degree and time of onset in different dogs and this probably affects the plasma volumes. For instance, dog 6 was studied through two cycles of insufficiency. The first time she was in insufficiency, the plasma volume was 25 per cent below the normal. The dog was then injected with hormone and realimented. Some time later she was again studied. However, the second time in insufficiency the dye method indicated a plasma volume decline of but 13 per cent. Presumably, the degree of insufficiency was approximately the same in both experiments.

Harrop et al. (1933) have recorded decreases in blood volumes of adrenalectomized dogs in insufficiency varying from 10 to 30 per cent. The volumes were calculated from hematocrit readings alone. Data obtained in such a manner would appear to have rather limited significance.

Dye disappearance curves obtained on both normal and adrenalectomized dogs in insufficiency indicate that the mixing time for the dye was apparently satisfactory.

Those animals having low initial hemoglobin show greater reductions of the plasma volume than animals with normal hemoglobin values. Compare dogs 7 and 5 with 12 and 11, figure 2. These animals (7 and 5) were taken off extract by gradually decreasing dosage over periods ranging from 4 to 8 weeks.

In concluding this section the writers wish to reiterate the opinion that the variations in the per cent of change from the normal of the 15 experimental animals studied, are probably due more to inadequacy of the dye method in adrenal insufficiency than to actual volume variation on such a wide scale. It appears likely, from volume data obtained by another method (Welcker's) and studies on "free bleeding" that the circulating volume in adrenalectomized dogs exhibiting marked symptoms is decreased by approximately 45 to 55 per cent (table 2).

B. *Blood volume of the adrenalectomized dog—Welcker method.* Kellaway and Cowell (1923) employed the Welcker technique and dye method on one adrenalectomized cat exhibiting symptoms. The dye method gave contradictory results whereas the Welcker method showed a significant reduction of the blood volume.

The modified Welcker technique of Smith, Arnold and Whipple (1921) was used in the present experiments. Extraction of the macerated tissues was not done. The perfusion fluid consisted of Locke's solution containing 1 per cent glucose and warmed to body temperature. Approximately one liter of fluid per kilogram of body weight was used; the duration of the perfusion varied in the different cases from 80 to 109 minutes. The animals were placed on their backs, the left femoral vein and right femoral artery cannulated under local anesthesia, and the dogs bled from the artery until no more blood would flow and the breathing had stopped.

The heart, however, continued to beat weakly. This blood as collected constituted the "free bleeding" (fig. 3). The warmed Locke's solution was then allowed to flow in through the femoral vein of the opposite leg under gravity pressure. The heart beat at once became much stronger and the respiration was resumed upon advent of the fluid. The heart continued to beat for varying periods of time in the different cases after the fluid entered. Both in the normal and in the adrenalectomized dog

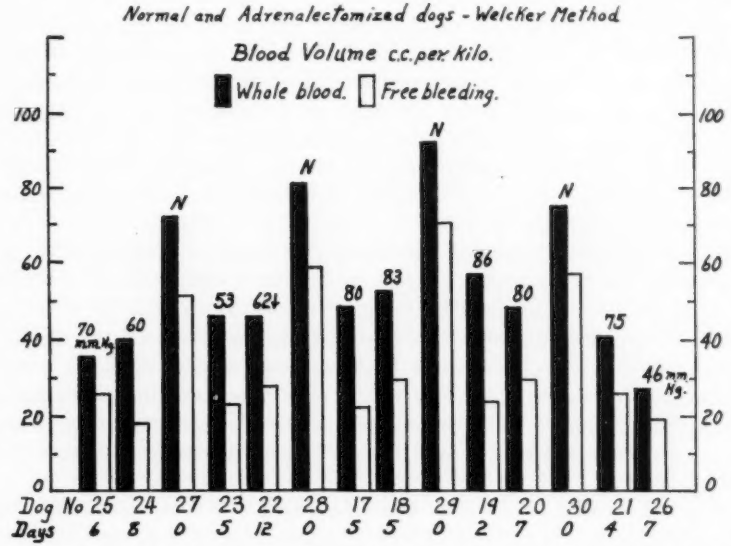

Fig. 3. The blood volumes (Welcker method) of four normal unoperated and ten adrenalectomized dogs not receiving hormone. *N* = normal control animals. Adrenalectomized dogs 17, 18, 19, 20 and 21 were used before any evident symptoms were present. The animals were eating full rations and appeared in normal health. Dogs 23 and 26 were exhibiting marked symptoms, dogs 22, 24 and 25 were in mild insufficiency. *Days* = number of days the animal was off extract before use in the experiment; numerals at top of columns = mean arterial blood pressure in millimeters of mercury at time the dogs were used.

it continued beating for 9 to 13 minutes after perfusion started. Some time before the perfusion fluid had all been used, the fluid flowing from the femoral artery was colorless. Edema developed during the experiment and always appeared earlier in the adrenalectomized than in the normal dog. The hemoglobin values of the blood obtained by "free bleeding" and those by artificial perfusion were separately determined.

At autopsy following perfusion, gross inspection of the animals showed them to be free of blood. Microscopic examination of the tissues was not

made. Aside from a few small areas of congestion in the liver and spleen, the tissues and organs were colorless.

The decline in blood volume and amount of blood obtainable by "free bleeding" of these animals as compared with the normal dog is evident (fig. 3). The plasma volumes were calculated from the hemoglobin values but are not recorded here since they involved using the hematocrit readings as the basis for the calculations. Table 2 summarizes the data obtained by the "washout" method.

Critical comment on the use of Welcker method in adrenal insufficiency. It is difficult to compare results obtained by the dye method with those obtained by using the Welcker technique, since the former is a plasma volume method and the latter a measure of the total red cell volume. We have previously stated reasons for the belief that the dye method is probably inadequate for accurate study of plasma volume in adrenal insufficiency, and it would appear that the "washout" method is not entirely satisfactory in this syndrome.

The adrenalectomized dog exhibiting symptoms, if killed quickly and autopsied, reveals marked congestion of the viscera. If the Welcker method for blood volume determination is employed and the animal is perfused until the perfusion fluid flowing from the femoral artery is colorless, it is reasonable to assume that many more corpuscles have been washed out of the animal by perfusion than were actually in circulation during insufficiency. The hemoglobin of these stagnant masses of noncirculating cells, however, enters into the calculations and swells the final volume figures.

It is on account of this fact that the amount of "free bleeding" may possibly give results which more closely approximate the actual amount of circulating volume of blood in adrenal insufficiency than either the dye or Welcker methods.

Roome, Keith and Phemister (1933) studied the average bleeding volumes, "free bleeding" of 50 anesthetized dogs after reducing the blood pressure to "shock levels" by various means. They observed that the bleeding volume was not dependent upon the initial head of pressure, but rather upon the actual volume of blood present in the circulatory system. When the blood pressure was depressed to the "shock level" by hyperventilation, anaphylaxis, histamine administration, spinal cord section or high spinal anesthesia, the average bleeding volume was but slightly diminished from normal. On the other hand, lowering of the blood pressure to similar levels by hemorrhage, trauma to an extremity, intestinal manipulation or plasmapheresis led to very considerable reduction of the bleeding volume from normal (e.g., 21.6 per cent of the calculated blood

volume as compared with an average of 58.6 per cent of the calculated blood volume obtained by "free bleeding" in 20 normal dogs).[3]

The data recorded in figure 3 show that the blood pressure level of the adrenalectomized dog off extract, and before the appearance of clinical symptoms, is not an adequate index of the extent of blood and plasma volume depletion. When the blood pressure has declined to 86 or 80 mm. Hg, the animal has already lost an average of 33.8 per cent of the circulating blood volume. The decline in bleeding volume, "free bleeding" at this level of arterial pressure is 56.3 per cent as calculated from the data in table 2. At this level of pressure the adrenalectomized dog is active, vigorous and eating full rations (fig. 3, dogs 17, 18, 19 and 20).

The general level, as to the amount of "free bleeding," is much the same whether the blood pressure registers 86 mm. Hg and the dog appears and behaves normal, or is 53 or 46 mm. Hg, and the animal verging on prostration from insufficiency. Dog 26 (fig. 3) exhibiting marked symptoms, and with the blood pressure at the shock level of 46 mm. Hg, showed but little less "free bleeding" than did case 17 with a blood pressure of 80 mm. Hg and free from evident symptoms, or case 24 with a blood pressure of 60 and exhibiting only slight symptoms. Compare, also cases 22, 23, and 25 with 19 (fig. 3). The explanation of this fact appears to be that when the critical level (86–80 mm. Hg) of arterial pressure has been reached, due to loss of blood volume, any further slight decrease in volume leads to drastic reduction of blood pressure and onset of severe symptoms. The data on free bleeding are summarized in table 2.

The blood volumes by the Welcker method (fig. 3 and table 2) check very well with the blood pressure findings when the critical level of pressure is taken into consideration. The pressure in insufficiency is dependent upon the volume changes. The critical level of pressure is the last defense of a failing circulation, due to loss of circulating fluid, and appears to represent the maximum degree of compensation which the heart and blood vessels are capable of undergoing in face of the declining blood volume. After the critical level of blood pressure has been reached, any further loss of blood may drop the mean arterial pressure as much as 1 mm. Hg for each cubic centimeter withdrawn.

[3] In comparing the present experiments with those of Roome, Keith and Phemister, it should be borne in mind that their experiments were acute, and performed on normal dogs, the entire series of events occupying a relatively short interval. The adrenalectomized dog off extract, on the other hand, develops insufficiency over a period of days, and therefore compensations and adjustments of the vasomotor system and changes in general muscular efficiency which may occur, have greater opportunity to manifest themselves in determining the amount of blood obtained by "free bleeding."

All of the animals showed a decreased plasma volume (dye method) when the blood urea level was significantly elevated. In two animals (dogs 12 and 16) an effort was made to directly correlate the blood urea nitrogen level and the plasma volume changes. Both of these animals showed a decreased plasma volume before the blood urea nitrogen was significantly elevated. For example, dog 16 (not included among the 15 animals in fig. 2) had a normal plasma volume of 378 cc. and a blood urea nitrogen of 32 mgm. per 100 cc. of blood. Two days after discontinuing extract injection the plasma volume had declined to 276 cc. and the urea had risen to 73 mgm. per cent. A similar relationship between blood volume and blood N.P.N. and urea nitrogen is shown in the case of dog 9, table 1.

Table 1 is of interest also in that the relation between decrease in blood and plasma volume is correlated with increases in blood and serum solids,

TABLE 2

Summary of blood volume changes in adrenalectomized dogs off extract compared with the normal. (Welcker method)

NORMAL 4 DOGS	ADRENALECTOMIZED BLOOD-PRESSURE 4 DOGS = 86–80	ADRENALECTOMIZED BLOOD-PRESSURE 6 DOGS = 75–46	ADRENALECTOMIZED BP.—TOTAL—10 DOGS 86–46 MM. HG	ADRENALECTOMIZED PER CENT OF CHANGE FROM NORMAL
Free bleedings (averages)				
cc. per kgm.	*cc. per kgm.*	*cc. per kgm.*	*cc. per kgm.*	
59.2	25.9	23.1	24.5	58.6
Whole blood (averages)				
80.4	53.2	33.3	43.2	46.2

water balance (i.e., total fluid intake and output) pulse rate, weight changes and blood glucose.

All of the available evidence from the Welcker blood volume determinations and the "free bleeding" as well as the dye method data, point to the conclusion that the greatest decline in blood volume of the adrenalectomized dog occurs during the first few days following extract withdrawal and before clinical signs of insufficiency appear. During this interval the dog is eating full rations and appears active and vigorous. Further evidence of an indirect nature for this conclusion is furnished by data (previously published) on the effect of slight hemorrhage in the adrenalectomized dog, and the study on water balance (Swingle, Pfiffner, Vars and Parkins, 1934).

The two factors responsible for the decline in blood and plasma volume and arterial pressure, and the general hemoconcentration and dehydration of adrenal insufficiency are: 1, the animal exhibits a negative water balance

and loses somewhat more fluid from the body than he takes in (owing to base loss by way of the kidney, Loeb et al., 1933; Harrop, Weinstein et al., 1933); 2, the normal fluid exchange between blood stream and tissues is upset. In the absence of the cortical hormone, the blood water slowly transudes into the tissues and interstitial tissue spaces and is immobilized so that the fluid shift from the tissues and interstitial tissue spaces does not occur. Following hormone injections the available fluid (and base) are mobilized and redistributed. The fluid and base shift is to the blood stream with consequent rise in blood volume, decrease in hemoconcentration and disappearance of clinical symptoms.

SUMMARY

1. The hemoconcentration of adrenal insufficiency is due to depletion of the blood and plasma volume. It is not merely a terminal event but is one of the primary and initiating factors in the syndrome. Studies of base and chloride changes were not made.

2. The plasma volume of 15 adrenalectomized dogs (16 expts.) was studied, using the dye (Congo red) method. Following withdrawal of cortical hormone, the plasma volume declines with increases in hemoglobin, cells by hematocrit and blood urea nitrogen. After hormone injections the plasma volume rises and the hemoconcentration disappears.

3. Clinical improvement of the animal parallels the increase in plasma volume and decrease in blood concentration.

4. The plasma volume of the 15 animals in insufficiency decreased from 13 to 56 per cent with an average of 33 per cent. Terminal plasma volume figures ranged from 20 to 46 per cent with an average decrease of 36 per cent. The greatest plasma volume changes were observed in a series of 6 dogs with low initial hemoglobin that had been adrenalectomized 4 to 12 months previously and brought into insufficiency by gradual reduction of hormone dosage over periods of 4 to 8 weeks. The average plasma volume decrease in this group was 39 per cent with a range of 25 to 56 per cent.

5. The blood volume (total red cell volume) of a series of 10 adrenalectomized dogs was studied by the Welcker (washout) technique. The animals were used for the experiments when the blood pressure stood at various levels. The average whole blood volume of 4 normal dogs was 80.4 cc. per kgm.; the average for 4 adrenalectomized dogs off extract and free from evident symptoms, with blood pressures varying in the different cases from 86 to 80 mm. Hg, was 53.2 cc. per kgm., the average for 6 dogs off extract with blood pressures varying from 75 to 42 mm. Hg was 33.3 cc. per kgm., the average for all 10 adrenalectomized animals was 43.2 cc. per kgm. representing 46.2 per cent decrease from normal.

6. The average amount of free bleeding of 4 normal dogs was 59.2 cc. per kgm., for 4 adrenalectomized animals off extract, but free from clinical

symptoms, and with blood pressures ranging from 85 to 80 mm. Hg, 25.9 cc. per kgm.; for 6 adrenalectomized dogs off extract with blood pressures ranging from 75 to 46 mm. Hg 23.1 cc. per kgm.; the average "free bleeding" volume for the series of 10 adrenalectomized dogs being 24.5 cc. per kgm., representing a 58.6 per cent decrease from normal.

7. Both the dye and Welcker methods of blood volume determination do not appear to be entirely satisfactory when employed in conditions such as adrenal insufficiency, where marked stagnation, and collapse of large areas of the circulation, and shunting of blood, occur.

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TISSUE METABOLISM OF WARM-BLOODED ANIMALS AT NORMAL AND AT FEBRILE TEMPERATURES

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The increase in oxygen consumption with rise in temperature has been studied rather extensively with both the intact animal and with the excised organs over the lower temperature ranges but comparatively few studies have been made of the effects of fever temperatures upon the excised tissues of warm blooded animals.

With the intact animal, however, the increment in metabolism with increase in temperature above normal has varied with the conditions under which it was studied. DuBois (1921) obtained a fairly uniform response in oxygen consumption in patients with various pathological fevers but attempts to raise the body temperature by extrinsic means, in either man or animals, have usually resulted in the most variable and often exaggerated increases. A large part of this, however, was due undoubtedly to the enormous increases in respiration which resulted (e.g., McConnell and Yagloglou, 1925; Nasset, 1932).

Recently, however, Nasset and Peters (1933) with amytalized dogs have succeeded in separating these two effects and while the responses to increased temperature were rather variable, yet the average result compared rather favorably with that of DuBois on fever patients.

Nevertheless a study of the effect of febrile temperatures upon excised tissue seemed worth while since it might throw some further light on the mechanism of the increased metabolism. If the rise were merely the result of increased chemical reactions, as DuBois believed, isolated tissue should show a rise approximately equal to that found in the fever patients, unless, of course, it should be found that different tissues gave unequal responses to rise in temperature.

METHOD. Two water baths were employed, one maintained at 37.5°C. and the other at the desired febrile temperature, which permitted the simultaneous determination, in duplicate, and more often in triplicate, of the metabolism at the two temperatures upon tissue slices from the same organ. Experiments were run with the second bath at 39.5°, 41° and 42°C.

The Warburg apparatus and, in the beginning, also the Warburg technic (1923) were used for the study. This method employed as a medium for the tissue a Ringer's solution buffered with a phosphate mixture to a pH of

7.4 and enriched by the addition of glucose to a concentration of 0.2 per cent. A second feature of the original technic was the equilibration of the tissue and apparatus against an atmosphere of pure oxygen just prior to its insertion in the water bath.

Such conditions, while adequate to maintain fairly well the oxygen consumption of tissue for a period of several hours at normal temperature, were quite inadequate when the tissue was subjected to febrile temperatures. At these temperatures oxygen consumption, higher at the beginning, of course, than that of the control tissue at 37.5°, fell so rapidly that often it reached the level of or even fell below that of the control. The increase in the metabolism due to increased temperature was, under these conditions, merely a function of the length of the determination.

In the endeavor to find conditions under which the metabolism at febrile temperature could be maintained for a period of hours, or at least maintained at a rate which would parallel the rate at 37.5°, several factors were found which influenced to a considerable extent the metabolism at the higher temperatures.

Oxygen vs. air. One of these factors was the percentage of oxygen which surrounded the tissues. When the bottles were left filled with air instead of oxygen, the metabolism, while usually lower, was *much more constant* over a period of hours.

It seemed probable that the lowered oxygen absorption in the air was the result of incomplete respiration, that the weight had included tissue which had contributed little or nothing perhaps to the oxygen uptake because the tension of oxygen had not been sufficiently high to allow penetration to the center of the tissue slices. This, however, was found to be true only in part. With thinner sections the metabolism was increased to some extent but still the average value of the oxygen consumption of tissues studied in air was lower than when studied in an atmosphere of pure oxygen. For example, in one experiment the oxygen consumption of a rat liver with sections about 0.5 mm. (the usual thickness for study in oxygen[1]) was 5.48 mm.^{3}/mgm. dry weight the first half hour, when measured in oxygen and 2.61 mm.^{3} when measured in air. Experiments with thinner sections (0.2–0.3 mm.) of different rats gave values ranging from 3.72 to 4.20 mm.^{3}/mgm./30 min. in oxygen and 2.62 to 3.55 mm.^{3}/mgm./30 min. in air, an increase from former figures in air but a decrease in oxygen. This latter is in accord with Warburg that there is an optimum thickness and that sections either above or below this optimum give lower values for oxygen absorption.

The fact, however, that not infrequently the metabolism fell off scarcely

[1] For tissue with a metabolism around 25 mm^3/hr./mgm. dry weight the critical thickness is 0.367 mm. and for tissues with a metabolism around 10 mm^3 it is 0.575 (Shorr, Loebel and Richardson, 1925).

at all over a period of three hours (usually not studied longer) and never more than 20 per cent in air, in contrast to a possible fall of 75 per cent in oxygen (see experiment of March 21, fig. 1) suggests that the results obtained under such a high tension of oxygen are perhaps not as accurate or at least not as nearly normal as are the values obtained at the lower oxygen tension.

When raised to 41° the decline in air was, in most cases, still greater than at 37.5° but there was not the great difference that there had been

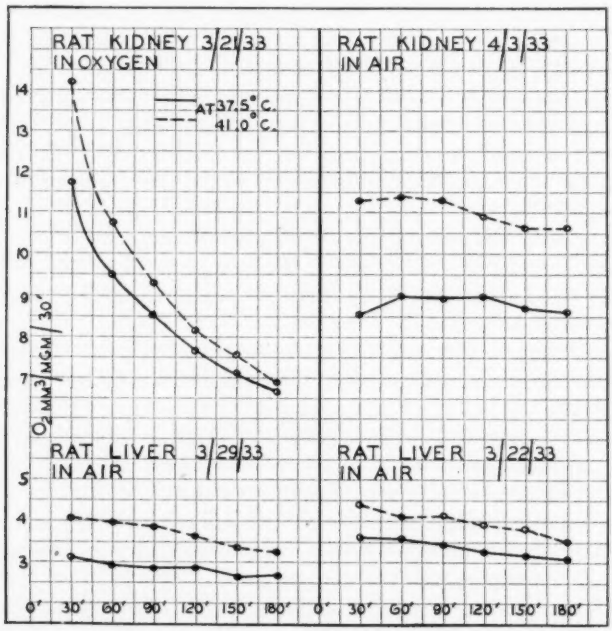

Fig. 1. O_2 absorption of fresh tissue in oxygen and in air. Ringer's-phosphate-glucose solution.

when studied in an atmosphere of oxygen. Compare experiment of March 21 in which the increase in oxygen consumption at 41° for the first half hour was 20.9 per cent and at the end of three hours was only 3 per cent, with experiments of March 22, 29 and April 3, in which the increase the first half hour as compared with the last was 22.1 per cent to 12.3 per cent; 30.9 per cent to 23.0 per cent; 32.5 per cent to 23.2 per cent respectively.

In these experiments the CO_2 was absorbed as it was produced by alkali within the bottle so the atmosphere was at all times free of CO_2. Pure oxygen is known to be injurious to the body, when breathed over any

considerable period, and it is possible that in these experiments, even in such a short period, it exerted a definite toxic action which was increased at the higher temperature.

Age of tissues. Previous work on tissue at 37.5°C. had shown that it was possible to keep the tissue at a temperature just above freezing for several days, 24 to 48 hours at least, and still have the metabolism essentially the same as in the fresh condition. More extensive experiments with older tissue disclosed the fact that often the initial height of the metabolism after storage was higher than it had been in the fresh stage and then declined much more rapidly, particularly at the higher temperature, as is shown in figure 2.

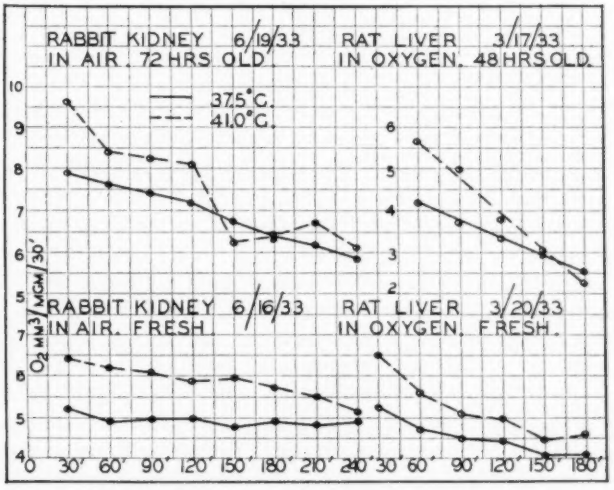

Fig. 2. O_2 absorption of fresh tissue and of old tissue at 37.5° and at 41°C. in Ringer's-phosphate-glucose solution.

Experiments of March 17 and March 20, 1933 are of rat liver studied in oxygen, after 48 hours' storage and in the fresh state respectively. Note that in the old tissue the metabolism at 41°, which in the first half hour was 35 per cent above the value at 37.5°, fell off so rapidly that at the third hour it was less than than that of the control tissue. This is in sharp contrast to the next experiment in which the tissue was fresh. The increase in this one however was not uniform but declined slowly.

In these two experiments it is not possible to compare the initial heights of the metabolism because the tissue is from two different rats but experiments of June 16 and June 19 are of the two kidneys of the same rabbit in air instead of oxygen. In the second in which the tissue is 72 hours old,

the initial oxygen absorption at 37.5° is 7.88 mm.³/mgm./30 min. as against 5.21 in the fresh state. During the four hour determination it fell off 25 per cent in the old tissue and only 5 per cent in the fresh state. At 41° the decline is more rapid in both but most rapid in the old tissue.

The reason for the increased metabolism in the old tissue is not clear. It was too large to be entirely bacterial in origin. In the short periods of the experiments bacteria undoubtedly played little or no part in the oxygen absorption.

The more rapid decline might possibly be caused by the production and accumulation during storage of a substance which is toxic to the tissue, to some extent at 37.5° and to a greater extent at 41°. It is perhaps possible that this same toxic product (if it exists) might act first as a stimulant when the temperature is suddenly raised from about 2° to 37.5° and 41°. But whatever may be the explanation it seems evident that for the study of fevers only fresh tissue should be used.

Blood serum. Although the results obtained with fresh tissue in air were more satisfactory than the early ones in oxygen had been, the increase in metabolism with the febrile temperature still fell off from reading to reading in many experiments. Consequently the next change made in in the conditions was the substitution of serum for the Ringer's-phosphate-glucose solution.

In two experiments using ox blood serum with rat tissue at 37.5° it was found that the metabolism was higher in the serum than in the Ringer's and more constant for 3 to 4 hour periods in air than in oxygen. In air and in serum the rate of oxygen absorption remained at nearly the same level throughout the period of observation at 37.5°, but at 41° fell so rapidly that in both cases, at the end of an hour and a half, it was down to the level of the control and fell continously through the whole three hour period of observation.

Since the serum used had been stored in the refrigerator for two or three weeks, it was possible that the sugar content had fallen so low that with the higher rate of oxidation at 41° the decline was due to exhaustion of the fuel supply. But if exhaustion of available sugar were the cause for the decline at the higher temperature one would expect the curve of the oxygen absorption to be rather different than it was. The decline was no greater the last hour than it was the first—in fact, rather the reverse.

It seemed more probable that the decline at 41° was due either to some toxic substance present in the foreign serum or to some product accumulated during storage, as was suggested in the case of the old tissue. No further work was done with foreign sera but a later experiment with fresh cat tissue in fresh and in old cat serum showed the fresh serum to be much superior to the old in maintaining the metabolism at its initial rate. The striking thing is the very pronounced effect (whatever its cause may be)

when the temperature is raised to 41° in contrast to little or no effect when the temperature is normal.

In figure 3 are charted a few experiments with rabbit kidney studied in fresh serum from the same animal.

In the first experiment (June 14,) there were no control determinations in Ringer's solution and the difference in the metabolism at the two temperatures was not as constant as in some of the other experiments. In the

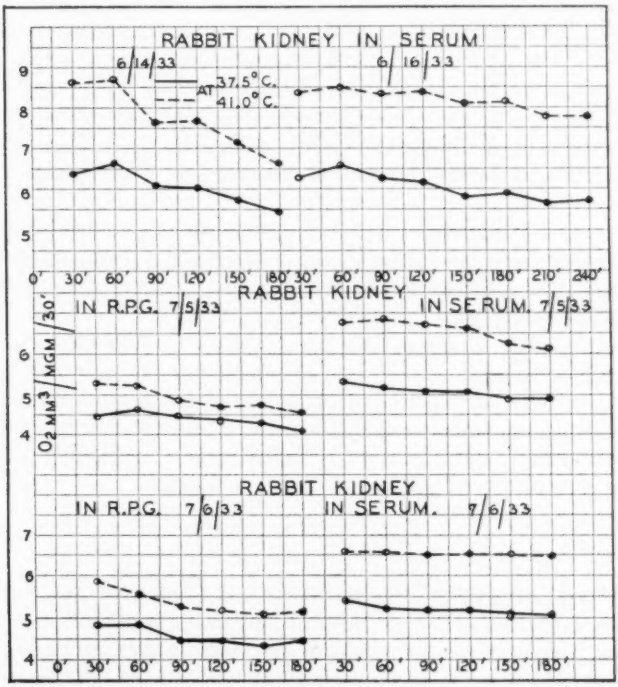

Fig. 3. O_2 absorption of fresh tissue in air, in blood serum vs. Ringer's-phosphate-glucose solution.

second experiment (June 16) the curves at the two temperatures were nearly parallel for a period of four hours and what change there was, was toward an increase rather than a decrease as the experiment progressed. The corresponding control in Ringer's is shown as the experiment of June 16, in figure 2. Note the difference in the two charts. In serum the initial increase was around 32 per cent and was maintained throughout while in the Ringer's it started a little lower, around 23 per cent, and fell to 5 per cent. The next two experiments (July 5 and 6, fig. 3) show essentially the

same thing, i.e., an increase at 41° which is definitely more uniform in serum than in Ringer-phosphate-glucose.

In all the experiments presented in figure 3, it happens that there was a greater increase of metabolism at 41° in the serum than in the Ringer's but this was not a constant feature of all the experiments and in at least two on cat kidney there was a greater increase in Ringer's than in serum.

A comparison of the experiment of March 17 (fig. 2) of old tissue in oxygen in Ringer's solution with the experiment of July 6 of fresh tissue in air and in serum is sufficient evidence of the superiority of the latter technic.

RESULTS. While it is only the later results (obtained in serum and air) which are of the most value, if the effect of fevers on tissue metabolism is to be compared with the effect on the intact body, yet the early ones (in Ringer's and in oxygen) are not without interest and are perhaps as valuable as the later ones for comparison of the different tissues from different animals.

In these first experiments, however, it must be remembered that the increases are average values of a diminishing quantity, in contradistinction to the results of the last experiments in serum and air. All values given in the tables are for the first hour only.

The tables which for the sake of brevity are of average values only, give the oxygen consumption, expressed as cubic millimeter per hour per milligram of dry tissue, at 37.5°C. and at the febrile temperature, together with the total percentage increase and the increase per degree difference in temperature and lastly the Q_{10} values, with the range of variability, calculated from the formula given by Bazett (1927) as follows:

$$\log Q_{10} = \frac{\log k_1 - \log k_2}{t_1 - t_2}$$

where k_1 and k_2 represent the metabolism at the higher and lower temperatures respectively and t_1–t_2 is the difference in temperature.[2]

All that is claimed for the results in the tables is that they represent the average values obtained in the number of experiments performed. The variation was so great, both in the initial oxygen consumption at 37.5° and in the response to the higher temperature, from animal to animal, that a larger number of experiments might change the averages given here by an appreciable amount. This variability in the rate at normal temperature is coming to be appreciated more than it perhaps was in the earlier reports of the oxidative rates of various tissues, and at least several factors are now

[2] The question as to whether the thermal increment is better represented by the Q_{10} value of Vant-Hoff or by the μ value of the Vant-Hoff-Arrhenius equation (1915) or by the empirical formula of Belehradek (1926) is of little significance here since the data presented are for a constant and very narrow range of 4.5°C. at most (37.5°C. to 42°C.).

known which influence the height of this metabolism. Wels (1925) found that it varies with the age of the animal from which the tissue was taken, being higher in the younger animal; sex probably makes a difference (Kagiyama, 1932; Stöhr, 1932); and in the case of the liver, the ratio of glycogen content to fat content makes a difference, high glycogen giving a higher oxidative rate (Hayashi, 1932). It may be possible that these factors also influence, at least to some extent, the response of the tissue to fever.

Table 1 shows what many other workers have shown, that the kidney tissue (in the rat) has an oxidative rate which is two to three times the rate

TABLE 1

Oxygen consumption in Ringer's-phosphate-glucose solution in oxygen (one hour periods)

TISSUE	NO. EXPTS.	TEMP.	O_2/MM.^{3}	INCREASE	INCREASE/1°	Q_{10} (range)
		°C.	*mgm /hr.*	*per cent*	*per cent*	
Rat kidney	11	37.5	21.0			
		42.0	26.2	25	5.5	1.6 (1.3–1.9)
	8	37.5	22.9			
		41.0	27.3	19	5.4	1.7 (1.5–1.9)
	3	37.5	21.3			
		39.5	23.7	12	5.8	1.8 (1.6–2.2)
Rat liver	5	37.5	9.2			
		41.0	11.0	18	5.1	1.6 (1.2–2.2)
	5	37.5	7.7			
		39.5	8.4	10	4.7	1.6 (1.3–2.0)
Rabbit kidney	7	37.5	10.2			
		42.0	11.8	16	3.6	1.4 (1.3–1.5)
	6	37.5	10.7			
		41.0	12.8	20	5.6	1.7 (1.4–2.1)
Dog kidney	2	37.5	12.5			
		42.0	15.3	23	5.1	1.6 (1.5–1.6)
	4	37.5	15.3			
		41.0	18.0	18	5.2	1.7 (1.3–2.0)

for the liver. Some of our unpublished work shows also the rate for rabbit liver to be considerably lower than the rabbit kidney values given in the table (approximately a half). Since the respiration of the rabbit kidney was only about half that of the rat kidney, it might be expected, on the basis of size and from the work of Wels (1925) that the kidney of large dogs would have a still lower rate than the rabbit, but such was not the case. The dog kidney, at least in six experiments, fell between that of the rat and the rabbit.

In spite of the differences in the initial height of the metabolism of the different tissues at normal temperature, there was little indication in these experiments that the percentage rise with rise in temperature was any greater in one than in another. With the exception of the rabbit kidney studied at 42°, the increase per degree difference in temperature averaged in each case between 4.7 and 5.8 per cent. Kidney and liver were the only two tissues studied and in the experiments in oxygen (table 1) the percentage increase and the Q_{10} for the rat liver tended to be slightly lower than for the rat kidney, but in air (table 2) they were exactly the same. It seems quite probable then that the response of the two tissues to fever is similar.

TABLE 2

Oxygen consumption for one hour periods at 41°C.

CONDITIONS	NO. EXPTS.	O_2—MM.³/HR./MGM.		INCREASE	INCREASE/1°	Q_{10}
		37.5°C.	41°C.			
Rat kidney						
				per cent	*per cent*	
R.P.G.* oxygen	8	22.9	27.3	19	5.4	1.7 (1.5–1.9)
R.P.G. air	4	21.6	27.9	29	8.3	2.1 (1.8–2.6)
Rat liver						
R.P.G. oxygen	5	9.2	11.0	18	5.1	1.6 (1.2–2.2)
R.P.G. air	20	6.3	8.2	29	8.3	2.1 (1.4–3.0)
Dog kidney						
R.P.G. oxygen	4	15.3	18.0	18	5.2	1.7 (1.3–2.0)
R.P.G. air	3	11.3	15.1	34	9.5	2.1 (2.0–2.4)
Rabbit kidney						
R.P.G. oxygen	6	10.7	12.8	20	5.6	1.7 (1.4–2.1)
R.P.G. air	11	8.6	10.3	20	5.6	1.7 (1.4–2.8)
Serum air	11	10.1	12.4	23	6.6	1.8 (1.2–2.6)
Cat kidney						
R.P.G. air	8	9.0	11.3	27	7.7	2.0 (1.2–2.9)
Serum air	9	11.1	13.9	25	7.1	1.9 (1.4–2.6)

* R.P.G. = Ringer's phosphate-glucose solution.

With the rabbit kidney 42° C. was possibly too high a temperature since all seven experiments showed a smaller total increase than was found at 41°. Evans (1923) found the maximum respiration for a guinea pig uterus to occur somewhere between 40° and 45°C. beyond which it declined rather

rapidly. He made no finer separation but it is probable that 42° is close to the limit of heat which a tissue can normally withstand and furthermore it is possible that the limit might be slightly different for tissues from various animals.

The average Q_{10} values in oxygen (table 1), if we omit those for the rabbit kidney at 42°, were all between 1.6 and 1.8—a rather close agreement amongst the different groups, although the two extremes in individual experiments were 1.2 and 2.2. They were on the whole definitely low. Of the total of fifty-one experiments only five showed a Q_{10} of 2.0 or above. If only the first half hour were taken instead of the hour, the values were somewhat higher, but still under 2.0 for the average. When studied in air, however, most of the values were appreciably higher, as shown in table 2.

In this second table are presented the average results of one hour studies at 41° C. of various tissues in Ringer's solution with air, instead of oxygen, and results on the kidney of rabbit and cat studied in serum with air. For the sake of comparison, those particular studies in Ringer's and oxygen made at 41°C. are also repeated in this table.

The oxygen consumption at 37.5°C. was usually a little lower in air than it was in oxygen, although for rat kidney there happened to be little difference in the averages. Possibilities as to the cause of this difference were discussed in a previous section.

In serum the oxygen consumption was higher than in Ringer's. This is in accord with the findings of others (Meyerhof and Lohman, 1926; Horn, 1930; Chang, Gerard and Shaffer, 1932), although the reason for it is not definitely established. Meyerhof was inclined to attribute it to the combustion of lactic acid present in the serum but Dickens and Šimer (1931) refuted this. Horn found that varying the amount of glucose made no difference; the increase was not proportional to the amount of sugar present. Chang, Gerard and Shaffer believed that the increase was due, in part at least, to the protein fraction.

At 41°C. the increases in respiration in Ringer's and in air were, on the whole, about as variable as they were in oxygen but, strangely enough, they were usually higher. Rat kidney averaged 8.3 per cent rise per degree of fever in air as against 5.4 per cent in oxygen; rat liver was 8.3 per cent in air as compared with 5.1 per cent in oxygen and dog kidney 9.5 per cent in air as against 5.2 per cent in oxygen. Rabbit kidney was the exception, showing exactly the same in air as in oxygen, i.e., 5.6 per cent. The Q_{10} values of course showed a corresponding increase from 1.7 to 2.1; 1.6 to 2.1 and 1.7 to 2.1 respectively for the three tissues given above.

The experiments with serum were done on rabbit and cat kidney since in these the tissue could be studied in serum from the same animal. With rabbit kidney the Q_{10} was 1.8, only slightly higher than in Ringer's and in cat kidney it was slightly less in serum than in Ringer's (1.9 compared with

2.0). This makes it appear probable that the Q_{10} for a short period is approximately the same in serum as in Ringer's. The chief value of serum is not that it gives a higher Q_{10} but that it gives a more constant value. It maintains the metabolism at the febrile temperatures at a more uniform rate for a period of hours.

The reason for the higher Q_{10} values in air and the more constant values over a long period in serum is not clear unless we are to conclude that a medium of fresh serum and air more nearly simulates normal conditions in the living body. At least the effect of febrile temperatures upon isolated tissue more nearly approaches the values obtained in the whole body under these conditions than when studied in an artificial medium of Ringer's solution and under a high tension of oxygen.

If there were a question of lack of proper oxygen diffusion in air, one should expect that the increase due to temperature would be less in air than in oxygen. If, in the case of the rat liver, for example, the reason for the rate being 6.3 mm.[3] in air at 37.5° instead of the 9.2 mm.[3] which it was in oxygen, was an insufficient oxygen tension to supply all the cells then, when the oxidative rate was increased, as it was at 41°, we should have, not a greater percentage increase than there was in oxygen, but a lesser one.[3] The actual findings were the reverse of this, i.e., an increase of 18 per cent in oxygen and of 29 per cent in air for the rat liver and corresponding differences for all tissues, except that of the rabbit kidney. That tissue failed to show any difference in the average of the experiments done, although at least one experiment was higher than any in oxygen, giving a Q_{10} of 2.8.

This rather wide variation in the values of Q_{10} seems to be the rule rather than the exception in most of the previous work. In 1915 Kanitz collected a considerable amount of data from many older workers on the oxygen consumption, as well as on many other physiological functions, of various animals. The individual variations were very great but the average Q_{10} values were fairly uniform (between 2 and 3) over the intermediary temperature ranges. Practically all work which has been carried on over a considerable range of temperature has shown higher Q_{10} values at the lower temperatures with decreasing values as the temperature is raised.

Work of Evans (1923) on guinea-pig uterus (only two experiments however) illustrates this point and is also an example of the effect of febrile temperatures on warm-blooded tissue. He studied the oxygen consumption from 15 to 45°C. and from the curve which he drew through his figures the following Q_{10} values may be calculated:

[3] The slightly increased diffusion at the higher temperatures would account for but a small fraction of the extra oxygen absorbed.

Temperature degrees	Q_{10}
15 to 25	2.9
25 to 35	2.0
30 to 40	1.8
37.5 to 40	**1.7**
35 to 45	1.3

The chief interest in connection with this work is the value of 1.7 obtained for the range 37.5 to 40° since it is essentially the value obtained on the liver and kidney when studied under the conditions which Evans used, i.e., Ringer's solution equilibrated with oxygen.

This value is definitely below the Q_{10} value of 2.3 which DuBois found for fever patients and might be interpreted as evidence of a real difference between the response of isolated tissue to febrile temperatures and that of the intact body to febrile disease, were it not for the fact that when studied under more favorable conditions the Q_{10} of tissue is raised. In serum and under a lower tension of oxygen as in air, or even in Ringer's solution, if the oxygen tension is kept down, the average Q_{10} is 2.0 which approaches sufficiently the value of DuBois to make any such interpretation unlikely.

SUMMARY

The response of liver and kidney tissue of rat, rabbit, cat and dog to febrile temperatures was studied by means of the Warburg apparatus and under varying conditions.

The average increase per 1° rise in temperature was fairly uniform in all the series under the same conditions. When studied in Ringer's phosphate-glucose solution and equilibrated with oxygen the Q_{10} values averaged 1.7 for the first hour but were of little significance because of the much more rapid decline in the metabolism at the higher temperature than at normal temperature.

When air was substituted for the pure oxygen and serum for the Ringer's solution the metabolism at febrile temperature was maintained for several hours at a rate which paralleled the normal rate and at a slightly higher rate than previously. Under these conditions the average Q_{10} was 2.0.

This latter value of Q_{10} compares more favorably with the Q_{10} found with intact animals and makes it appear probable that isolated tissue responds to increased temperature with about the same order of intensity as does the body as a whole.

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THE RESPONSES TO CHANGES IN ENVIRONMENTAL TEMPERATURE AFTER REMOVAL OF PORTIONS OF THE FOREBRAIN[1]

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The investigations of Isenschmid and Krehl (1912), Isenschmid and Schnitzler (1914), Bazett and Penfield (1922), Keller and Hare (1932), and Bazett, Alpers and Erb (1933), have shown that the hypothalamic region of the forebrain contains either nerve centers or neural connections which are indispensable for the regulation of body temperature. That certain portions of the forebrain above this level play a rôle in this regulation, however, has been suggested by the work of Wood (1878), Aronsohn and Sachs (1885), White (1890), and others.

During the course of other investigations made in this laboratory (Bard, 1933), it was noted that cats, after the removal of the cerebral cortex, frequently shivered at ordinary room temperatures. This suggested the possibility that the cortex normally exerts an influence on the shivering mechanism, and led to an investigation of temperature control in animals which had survived for long periods the removal of varying amounts of the cerebral hemispheres. Dogs were used primarily, as their reactions to temperature changes are more constant than those of cats.

METHODS. The operative technique was essentially the same as that described by Bard (1933). The anesthetic of choice was pentobarbital sodium, intraperitoneally (0.65 to 0.70 cc. per kgm. for dogs; 0.75 cc. per kgm. for cats). The bilateral operations were done in two stages, with a sufficient interval for the animals to recuperate from the first stage before the second was performed.

No special care of the animals was necessary after unilateral lesions of the hemispheres other than to maintain their body temperature artificially until recovery from anesthesia. After the second operation, however, constant attention was required for several days, largely to protect the animal from injury during the excitement stage, and to provide fluids. As the anesthesia wore off, temperature control returned sufficiently to

[1] This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

permit keeping the animals under ordinary conditions. Dogs 10 and 11 were housed for a time in a room at 23° to 28°C., as the higher temperature seemed to lessen their overactivity. Most of the animals regained their ability to eat and drink sometime after the second operation, but dogs 10 and 11 and cat 228 had to be hand-fed during the remainder of their existence.

For study of their responses to cold the animals were placed in a refrigerated room, or occasionally, weather and temperature being suitable, out-of-doors, for periods of time ranging from 30 minutes to 2.5 hours. The temperatures varied from 2° to 15°C., but were constant within 1°C., during a single set of observations. Dogs were used exclusively in tests of the responses to heat. They were placed, unleashed, in a large, insulated room which was heated to between 36° and 41.5°C. by means of racks of resistance units. The temperature of the room was controlled by a thermostat and remained constant within 2°C. during any one set of observations.

The body temperature of the dogs was determined by inserting a clinical thermometer in the rectum to a depth of 5 to 6 cm. The readings were made at the end of three minutes. In the case of the cats the thermometer was held snugly in the inguinal region, with the animal's thigh pressed firmly against the abdominal wall. The readings were made at the end of 6 to 7 minutes. In a number of experiments surface-temperature records were also obtained by the use of thermocouples and a portable galvanometer. In some instances the thermocouples were kept continually in place on shaved areas of the skin by means of adhesive tape; in others the Benedict (1919) type of skin thermocouple was employed and held against the skin for from 15 to 20 seconds at each determination. These observations were from regions of the back 4 to 6 cm. on each side of the midline, at about the level of the 12th pair of ribs.

The brains of dogs 5, 10 and 11 were fixed in formalin, imbedded in paraffin and cut serially at 30μ. Every 5th section was mounted and stained with gallocyanin-chromalum (Einarson, 1932).

INDIVIDUAL RECORDS OF EXPERIMENTAL ANIMALS. Dog 5, young male, medium size, short hair. First operation, left side, July 28; second, right side, November 22, 1932. The dog remained in a fairly good state of health until April, 1933, when signs of failing began to appear. During the last months it was thin in spite of a large food intake, and had numerous skin lesions. Died on April 27, after a low spinal cord transection. A dorsal view of the brain is presented in figure 1. Histological examination showed the following: *Neopallium:* entirely removed except a fragment of the G. splenius posterior. *Rhinencephalon:* the olfactory stalk is divided and the tuberculum olfactorium injured rostrally on either side. The pyriform lobe and amygdaloid complex are slightly injured laterally and the septal nuclei dorsally. The dorsal part of the hippocampus has been destroyed and the fornix cut. *Striatum:* the head of the caudate is involved dorsally and laterally,

more on the left than the right. The putamen is extensively damaged on the left, only slightly on the right. The globus pallidus and entopeduncular nuclei are intact. *Thalamus:* not directly involved, but shows the typical degeneration which follows decortication. *Hypothalamus:* intact. The more caudal portions of the brain, e.g., the peduncles, show only secondary degenerative changes.

Dog 10, young female setter, medium size, long hair. Operation on right side June 6; on left side September 16, 1933. Recovery from the second operation was in general satisfactory, aside from an unusually prolonged inability to walk. Its condition was excellent until December, when it began to fail. It was killed January 13, 1934. The dorsal view of the brain is presented in figure 2. Histological examination showed the following: *Neopallium:* removed, except a few fragments bordering the septum and hippocampus. *Rhinencephalon:* the olfactory stalks are present, but damaged dorsolaterally. The tuberculum olfactorium, pyriform lobe and amygdaloid complex are damaged laterally on both sides, left more than right. A large part of the hippocampus, with the subiculum and praesubiculum, remains on either side, but the fornix is destroyed. *Striatum:* the caudate and putamen are moderately damaged on either side. *Thalamus* and lower portions of the brain show changes similar to those in dog 5.

Dog 11, small female Irish terrier. Operation on right side June 14, and on left September 15, 1933. The animal was unusually active after the second operation and injured its head. This resulted in a severe subcutaneous infection which healed under treatment. The dog was in good condition until early in November, when it began to lose weight, although there was no decrease in its appetite. Throughout its survival this animal walked about almost continually. It became quiet only when exposed to heat and during the few hours out of each twenty-four when it slept. It was found dead in the room on November 21. Autopsy disclosed some indication of respiratory infection. The dorsal view of the brain is presented in figure 3. Histological examination showed the following: *Neopallium:* entirely removed. *Rhinencephalon:* on the left there is only a small remnant of the tuberculum olfactorium. On the right the caudomedial half of the tuberculum olfactorium, the medial third of the amygdaloid complex and a fragment of the hippocampus remain. *Striatum:* on the right the ventromedial corner of the putamen, together with the globus pallidus and entopeduncular nucleus is seen. *Thalamus:* on the right the lesion involves the anterior and midline groups of nuclei rostrally, but leaves the habenular ganglion and the caudal nuclei of the medial group intact caudally. On the left all the dorsal thalamus is destroyed except the commissural portion of the ventral nucleus. *Hypothalamus:* intact aside from injury to the lateral margin of the lateral hypothalamic area and the rostrolateral margin of the zona incerta. The more caudal portions of the brain show secondary degenerative changes as in dogs 5 and 10.

Dog 6, young male, small, short-haired. The motor-sensory area of the cortex was removed February 20, on the left side, and April 14, 1933, on the right. The animal remained in excellent condition until sacrificed on June 10, 1933. The dorsal view of the brain is shown in figure 4.

Dog 7, young German police puppy. The left cerebral cortex was removed on April 7, 1933. Dorsal and lateral views of the brain are given in figures 5 and 6.

Dog 8, long-haired female, medium size. Right cerebral cortex removed on June 17, 1933. This animal has been kept alive for additional studies.

The cats used in the present experiments were prepared by Bard primarily for other purposes. Descriptions of certain characteristics of these

animals together with histological reports on their brains are given elsewhere (Bard, 1933, 1934).[2] The cerebral ablations carried out in cats 103 and 313 resembled those in dogs 5 and 10. The periods of survival of these animals were twenty-eight months and six weeks, respectively. Cat 244 was kept eleven months after removal of all neocortex, much of the rhinencephalon, both caudate nuclei and all the rest of the striatum on the

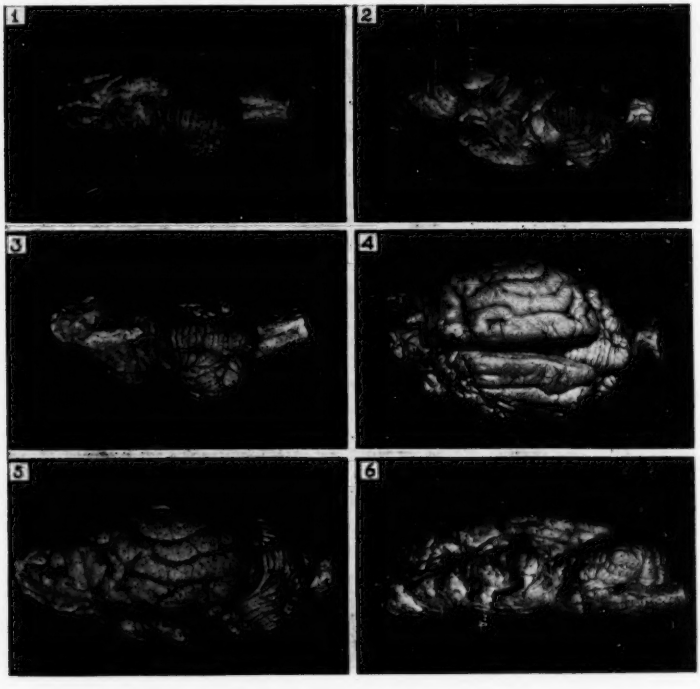

Fig. 1. Dorsal view of the brain of dog 5.
Fig. 2. Dorsal view of the brain of dog 10.
Fig. 3. Dorsal view of the brain of dog 11.
Fig. 4. Dorsal view of the brain of dog 6.
Fig. 5. Dorsal view of the brain of dog 7.
Fig. 6. Lateral view of the brain of dog 7.

left except a few cells of the globus pallidus and entopeduncular nucleus. Although the hypothalamus remained intact the greater part of the dorsal thalamus had been ablated. Cat 228 lacked all cerebral tissue above the

[2] A detailed report (Bard and Rioch) correlating the anatomical with the physiological findings in these animals will soon appear.

hypothalamus except the most medial part of the dorsal thalamus, a few cells of the left globus pallidus and small rests of the rhinencephalon (n. accumbens, ventrocaudal portion of septum, a caudomedial portion of one tuberculum olfactorium). The animal survived in good health for twelve months.

TABLE 1

Observations on normal dogs in cold-room

EXPERIMENT NUMBER	ANIMAL NUMBER	BASAL RECTAL TEMPERATURE	AVERAGE TEMPERATURE OF COLD-ROOM	MINUTES BEFORE SHIVERING BEGAN	RECTAL TEMPERATURE WHEN SHIVERING BEGAN	TYPE OF SHIVERING	TOTAL TIME IN MINUTES SPENT IN COLD-ROOM	RECTAL TEMPERATURE AT END OF STAY IN COLD-ROOM
		°C.	°C.		°C.			°C.
7	3	38.77	9.9	95.0	38.44	Vigorous	190.0	38.61
8	5	38.55	9.8	95.0	38.28	Average	182.0	38.50
10	5	38.74	9.8	105.0	38.11	Vigorous	156.0	38.22
14	6	38.25	3.1	10.0	38.66	Vigorous	64.0	38.95
30	8	38.80	8.9	52.0	38.75	Slight	74.0	38.75
35	9	39.20	4.1	36.0	38.80	Vigorous	110.0	38.65
42	10	38.75	4.2	48.0	38.85	Vigorous	105.0	38.55
44	11	38.05	5.9	18.0	37.85	Vigorous	84.0	37.65
63	"Mack"	38.41	5.5	55.0	37.84	Slight	60.0	37.84
Averages.....		38.61	6.8	57.0	38.40		113.9	38.41
Observations on dogs of group A								
15	5	39.00	2.9	7.0	39.10	Very vigorous	66.0	38.95
18	5	38.44	13.8	3.0	38.44	Very vigorous	169.0	38.50
20	5	38.69	10.2	*	38.72	Very vigorous	53.0	38.66
25	5	38.66	8.5	1.0	38.66	Very vigorous	45.0	37.95
27	5	37.84	15.0	*	37.84	Very vigorous	150.0	38.33
55	10	38.69	6.0	3.0	38.77	Very vigorous	63.0	38.95
57	10	38.47	5.5	*	38.47	Vigorous	65.0	38.89
59	10	38.47	6.1	*	38.47	Vigorous	47.0	38.77
56	11	39.01	5.7	*	39.01	Very vigorous	67.0	39.06
58	11	39.03	6.5	*	39.03	Very vigorous	55.0	38.89
60	11	38.83	6.4	*	38.83	Very vigorous	46.0	38.89
64	11	39.00	5.9	3.0	39.17	Very vigorous	58.0	39.06
Averages.....		38.68	7.7	1.4	38.71		73.7	38.74

* Shivering at ordinary room temperature.

PHYSIOLOGICAL OBSERVATIONS. All the animals with bilateral lesions involving the whole neopallium or more, i.e., dogs 5, 10 and 11, cats 103, 228, 244 and 313, showed entirely similar responses to changes in the environmental temperature, and in this respect form a group. For convenience, they will be referred to as animals of group A. The other

operated animals, including those with unilateral hemisphere ablations and partial removals of the cortex, may be classed as group B.

1. *Shivering.* The animals of group A frequently shivered at ordinary room temperatures. That this was true thermal shivering was indicated by its prompt disappearance when the animal was moved to a warm room (38° to 40°C.).

In tables 1 and 2 are summarized the observations on normal controls and on the animals of group A when exposed to low environmental temperatures. Dogs 5, 10 and 11 were first studied in the normal state as

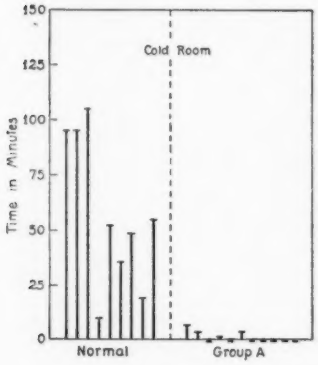

Fig. 7

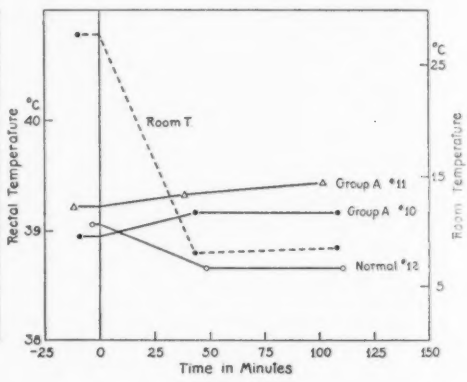

Fig. 8

Fig. 7. Graphical representation of the latent period for shivering in normal dogs and dogs of group A. The length of the lines (ordinates) represents the time in minutes before shivering started in different observations on several animals.

Fig. 8. Rectal temperature changes of a normal dog, 12, and of two dogs of group A, 10 and 11, when exposed simultaneously to cold. Abscissae: time in minutes; ordinates: temperature in degrees Centigrade.

controls, as well as after operation. The characteristic differences between these two groups were as follows:

a. The latent period before shivering began was greatly decreased in group A, more so in the dogs than in the cats. This pronounced difference is shown graphically in figure 7.

b. In 7 of 9 experiments on the control dogs shivering did not begin until after a drop in the rectal temperature. In the dogs of group A shivering began before any drop in rectal temperature occurred. The cats showed considerable variation in this regard.

c. The rectal temperature at the close of the period of exposure was lower than the basal rectal temperature in 8 of 9 cases in the control dogs. In the dogs of group A it was higher in 8 of 12 cases. The cats were again more variable.

d. As far as could be noted by general observation, the shivering of the animals of group A was vigorous and continuous throughout the period of exposure. In the controls the muscular twitches seemed much less forceful and the shivering was intermittent.

Temperature curves for individual experiments on normal dogs and dogs of group A are shown in figures 8 and 9, B, C, D and E. In the experiment shown in figure 8 a normal dog and two dogs of group A (10 and 11) were placed in a room which was at a temperature somewhat above average. At the end of an hour the basal rectal temperatures were taken and the room was immediately cooled to approximately 8°C. by opening all the windows. Dogs 10 and 11 promptly began to shiver vigorously, but the control only shivered slightly. As was usual with animals of group A, dog 11 showed considerable activity by walking about the room. This overactivity may account partly for the rise in temperature observed in the group A animals. Dog 10, however, was less active than the normal dog, so that at least in this case the rise must be accounted for solely on the basis of the temperature-control mechanisms.

The temperature curves for the normal dogs in figure 9, B, C, D and E, show that there was often a slight rise in body temperature immediately after exposure to cold. This was routinely followed by a decline, however, with a final temperature below the basal.

The difference in the duration of the latent periods for shivering in the normal controls could not be correlated with any factors observed. In order to see whether any consistent relationship between body temperature and occurrence of shivering could be observed in animals of group A, the following experiment was performed. Dog 10 and a normal female of approximately equal body size and hair covering were placed in a warm-room (35.5° to 38.5°C.), until a distinct rise in rectal temperature occurred. They were then directly transferred to a temperature of 17.0°C. Dog 10 began to shiver within 1 minute, although its rectal temperature was 39.72°C., whereas the normal dog, with a rectal temperature of 39.22°C., did not shiver at all during the 14-minute period of exposure.

Diurnal variations in body temperature at ordinary room temperature were found to be relatively small in both normal and group A animals. This is illustrated in figure 9A, the record covering a period from 10 a.m. to about 4 p.m. The variations are not large enough to modify the interpretation of the records of the experiments on exposure to cold or heat.

A number of observations were made on animals after the first stage of the operation, i.e., with unilateral ablations of the hemisphere (group B). The shivering was more vigorous than in the controls, and it is noteworthy that it was entirely symmetrical. In other respects the results were not consistent. In 2 of 4 experiments on dogs the latent period for shivering was shortened, in 2 there was no definite change. Rectal temperature

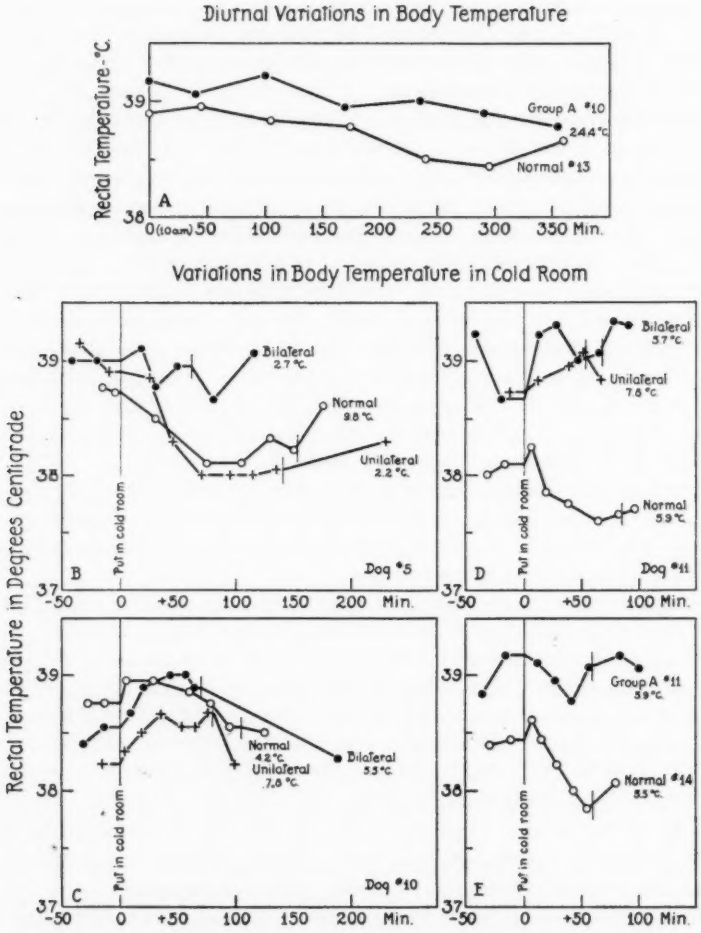

Fig. 9. Rectal temperature changes observed in dogs. Abscissae: time in minutes; ordinates: rectal temperature in degrees Centigrade.

A. Control experiment for diurnal variations at ordinary room temperature. Dog 10 and normal dog 13.

B, C, D. Exposure to cold of dogs 5, 10 and 11, respectively, before operation, *normal*, after the first stage, *unilateral*, and after the second stage, *bilateral*.

E. Dog 11 and a normal dog, 14, of similar size and hair covering, exposed to temperatures of 5.9° and 5.5°C. respectively.

curves for dogs 5, 10 and 11, during the unilateral state, are shown in figures 9B, C and D.

TABLE 2

Observations on normal cats in cold-room

EXPERIMENT NUMBER	ANIMAL NUMBER	BASAL RECTAL TEMPERATURE	AVERAGE TEMPERATURE OF COLD-ROOM	MINUTES BEFORE SHIVERING BEGAN	RECTAL TEMPERATURE WHEN SHIVERING BEGAN	TYPE OF SHIVERING	TOTAL TIME IN MINUTES SPENT IN COLD-ROOM	RECTAL TEMPERATURE AT END OF STAY IN COLD-ROOM
		°C.	°C.		°C.			°C.
2	307	38.28	8.4	120.0	38.11	Slight	120.0	38.11
7	381	38.89	6.0	95.0	38.66	Slight	340.0	38.77
8	377	37.78	6.7	*			185.0	37.22
11	374	37.84	6.3	210.0	38.61	Slight	256.0	38.83
12	374	38.55	8.1	68.0	38.72	Slight	283.0	38.66
16	374	37.44	7.2	45.0	38.28	Slight	233.0	38.28
19	384	38.77	6.7	144.0	38.66	Slight	157.0	38.66
20	302	38.77	6.7	67.0	39.00	Slight	142.0	39.06
21	303	38.77	6.7	50.0	38.55	Slight	172.0	38.28
22	300	38.89	6.7	*			182.0	38.06
23	304	38.11	7.6	142.0	38.77	Slight	142.0	38.77
30	304	38.44	2.2	10.0	38.89	Vigorous	55.0	39.22
33	306	37.66	9.0	90.0	37.84	Slight	181.0	38.11
35	304	38.72	9.6	70.0	38.50	Fairly vigorous	190.0	38.72
Averages....		38.35	7.0	92.6	38.55		188.4	38.48
				Observations on cats of group A				
1	228	38.66	8.3	†	38.72	Very vigorous	70.0	39.33
3	228	39.61	9.7	5.0	39.61	Very vigorous	165.0	39.61
4	103	38.72	9.7	35.0	38.66	Fairly vigorous	155.0	38.89
5	103	38.50	0.6	23.0	38.44	Very vigorous	88.0	38.55
6	228	38.22	6.0	†	38.33	Very vigorous	350.0	39.44
10	228	39.10	6.4	†	38.89	Very vigorous	245.0	39.44
15	228	39.06	6.2	†	39.06	Very vigorous	243.0	39.06
17	103	38.39	7.2	42.0	38.83	Vigorous	227.0	39.17
26	244	38.87	9.0	†	39.00	Vigorous	237.0	38.33
29	244	38.72	2.1	†	38.72	Vigorous	59.0	38.17
34	244	38.55	8.9	42.0	38.00	Vigorous	183.0	38.39
Averages....		38.76	6.7	29.4	38.75		183.8	38.94
				Observations on cats of group B				
9	237	39.44	6.7	15.0	40.00	Very vigorous	192.0	40.00
14	237	39.39	8.2	42.0	39.33	Fairly vigorous	290.0	39.50
Averages....		39.41	7.4	28.5	39.66		241.0	39.75
13	224	39.83	8.0	46.0	39.10	Slight	285.0	39.39

* No shivering occurred.

† Shivering at ordinary room temperature.

Three animals of group B were studied in an attempt to determine the relation of the motor-sensory area of the cerebral cortex to shivering. On dog 6 tests were made before and after bilateral removal of the motor-sensory area. There was no significant decrease in the latent period, but shivering became more vigorous after the bilateral lesion. In 2 cats, 224 and 237 (see Bard, 1933), an extensive removal of the hemisphere was performed on one side. On the other side in 224 all the cortex except the motor-sensory area was removed, and in 237 the motor-sensory area was removed but the remainder of the cortex was left intact. The observations made on the animals are shown in experiments 9, 14 and 13 in table 2. Cat 224 responded to cold as a normal cat, whereas 237 resembled the cats of group A.

2. *Fever.* Although it has been shown by numerous investigators that experimental fever can be produced in warm-blooded animals after complete removal of the cerebral hemispheres, it was considered worth while to study such a reaction, especially that part involving shivering, in an animal of group A. The pyretogenic agent employed was typhoid-paratyphoid vaccine, given intravenously. During the febrile reaction it was observed that though the operated dog (no. 10) shivered much more frequently and vigorously than did the normal dog its temperature rose less. It was difficult to compare accurately the shivering responses, however, because the group-A dog was shivering occasionally even before the vaccine was injected. In the normal dog the temperature rose from 38.72° to 40.73°C., in the group-A dog from 39.10° to 40.11°C.

3. *Surface temperature.* It was noted routinely that, immediately after the removal of one cerebral hemisphere or of the motor-sensory area on one side, the contralateral side of the body was appreciably warmer to touch. No accurate measurements were made, however, until two to four weeks after the operation. By that time the difference of temperature on the two sides in a number of the dogs had disappeared. A few animals, however, continued to show the change for long periods; e.g., dog 8, four months after removal of the right cerebral cortex, had a skin temperature of 32.67°C. on the right side and 33.36°C. on the left. Localization of this phenomenon to the motor-sensory area of the cortex is suggested by experiments on dog 6. Twenty-eight days after the first operation (ablation of left motor-sensory area), the skin temperature on the right was 35.94°C., on the left 35.06°C.

More prolonged than the differences of temperature on the two sides was the decrease of reflex vasoconstriction on the side opposite the cerebral ablation, when the animal was exposed to cold. In this respect also there was gradual recovery. For example, dog 7, with a left-sided ablation, reacted as follows when exposed in the cold-room. Four weeks after the operation the temperature on the left side dropped 4.80°C., on the right

3.77°C.; two weeks later, the left side 4.90°C., the right 4.68°C.; ten days later, the left side 3.79°C., the right side 3.73°C. In the group-A dogs the average drop of surface temperature on exposure to cold was less than that in the normal dogs. There was, however, in individual experiments considerable variation. This was largely dependent on differences in the initial room temperature and in the temperature of the cold-room.

TABLE 3

Observations on normal dogs in warm-room

EXPERIMENT NUMBER	ANIMAL NUMBER	BASAL RECTAL TEMPERATURE	AVERAGE TEMPERATURE OF WARM-ROOM	MINUTES BEFORE PANTING BEGAN	RECTAL TEMPERATURE WHEN PANTING BEGAN	TYPE OF PANTING	TOTAL TIME IN MINUTES SPENT IN WARM-ROOM	RECTAL TEMPERATURE AT END OF STAY IN WARM-ROOM
		°C.	°C.		°C.			°C.
2	3	39.22	39.0	2.0	39.88	Rapid	85.0	39.50
5	3	39.10	39.6	1.0	39.10	Rapid	70.0	39.00
3	5	38.66	38.9	2.0	39.33	Rapid	90.0	38.89
4	5	38.83	39.6	2.0	39.39	Rapid	70.0	39.00
29	8	38.25	39.6	*	38.25	Rapid	250.0	38.80
33	9	38.80	40.1	*	38.80	Rapid	105.0	38.80
41	10	38.95	40.2	1.0	38.95	Rapid	73.0	38.95
45	11	38.57	39.8	2.0	38.55	Rapid	84.0	38.77
16	6	38.50	36.0	2.0	38.50	Rapid	120.0	38.40
78	13	38.47	37.4	3.0	38.50	Rapid	138.0	38.72
Averages....		38.73	39.0	1.5	38.92		108.5	38.88
Observations on dogs of group A								
13	5	39.55	39.0	55.0	41.10	Slow	110.0	41.00
21	5	38.40	39.5	†			93.0	39.20
28	5	38.00	40.0	†			236.0	40.30
65	10	38.55	41.0	30.0	39.17	Slow	80.0	39.39
68	10	38.69	41.4	15.0	38.89	Fairly rapid	69.0	39.39
79	10	38.97	37.5	170.0	39.88	Slow	186.0	39.88
66	11	38.86	41.0	47.0	40.00	Slow	70.0	40.11
68	11	38.88	41.2	54.0	39.77	Slow	96.0	40.11
Averages....		38.80	40.1	62.0‡	39.80‡		117.0	39.92

* Panting at ordinary room temperature.

† No panting observed during stay in warm-room.

‡ Omitting cases in which no panting occurred.

4. *Panting.* In table 3 is given a summary of the observations made on 8 normal controls and on the 3 dogs of group A (5, 10 and 11). The differences of response of the two groups may be classified as follows:

a. Normal panting did not occur in the group-A dogs at the temperatures

employed. In contrast to the shallow polypnea of the controls the dogs of group A showed a slow and deep type of hyperpnea.

b. Panting occurred in the normal dogs after a very short latent period, which averaged 1.5 minutes, and at the longest was 3 minutes. In the dogs of group A the latent period before a change in respiration was noticeable was much longer, averaging 62 minutes. On two occasions dog 5 showed no increase in rate of respiration. The contrast is shown in figure 10.

c. In seven of ten experiments the controls began panting before any rise in rectal temperature had occurred. In all the group-A dogs the rectal temperature rose before respiration changed. This rise in temperature averaged 1°C.

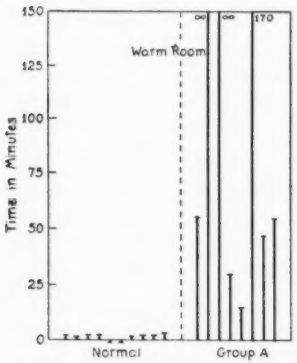

Fig. 10. Graphical representation (see fig. 7) of the latent period for panting in normal dogs as contrasted with that for hyperpnea in dogs of group A.

d. In only one case among the controls did the rectal temperature at the end of the period in the warm-room show a rise of more than 0.3°C. above the basal level. In four cases there was either no rise or actually a fall. In the dogs of group A the rise in rectal temperature during the period of exposure to heat averaged 1.12°C.

In experiment 68, on dog 10, not only were the respirations more rapid than in the other experiments on the group-A dogs, but the latent period also was shorter, and the rise in rectal temperature less. The average temperature of the warm-room was only 0.4°C. higher than in experiment 65, when this animal responded typically as a group-A preparation. This small difference in the environmental temperature would hardly appear adequate to explain the greater response. It should be noted that this animal had a less extensive lesion than any other in group A.

No doubt many of the observations on the dogs of group A would have been more conclusive had they been kept in the warm-room for longer periods of time. Since such animals are difficult to prepare it was deemed inadvisable to risk more prolonged exposures to heat.

DISCUSSION. In the preparations having the most extensive lesions in the present series the hypothalamus and the caudal third of the medial portion of the dorsal thalamus were not involved. The striatum was largely removed in dog 11 and almost completely in cat 228. All the rhinencephalon except the medial half of the tuberculum olfactorium and a fragment of the septum was removed in the same animals. In cat 244

the anterior two-thirds of the dorsal thalamus, including the midline nuclei, were removed or destroyed. Since temperature control in these preparations was similar to that in the preparations with the less extensive lesions it may be concluded that, in the absence of the neopallium and fornix, the following structures play no significant rôle in temperature regulation, namely, the striatum, the lateral basal olfactory areas and, possibly, the rostral two-thirds of the midline groups of thalamic nuclei. It is also apparent that the changes in the heat-control mechanism shown by the animals of group A are dependent on the removal of the neopallium. For purposes of localization this does not distinguish between the neopallium and the dorsal thalamus, since chronically decorticate animals show practically complete degeneration of the lateral group and of the lateral portions of the anterior and ventral groups of thalamic nuclei, moderate degeneration of the medial group and relatively slight degeneration of the midline group (cf. Nissl, 1913, and Holmes, 1902).

All the animals of group A were able not only to maintain a normal body temperature under average conditions, but also to withstand rather extreme changes in environmental temperatures for periods of several hours with relatively small changes in body temperature. The resistance to high environmental temperature was, however, much poorer than that of these same animals before operation. On the one hand there was a chronic vasodilatation, as shown by the surface temperature observations; on the other, instead of prompt, normal panting, a slow type of hyperpnea appeared only after the rectal temperature had risen about 1.0°C. In striking contrast was the excessive response to cold. Although there was apparently a failure of the peripheral vessels to constrict normally, the shivering mechanism came into play immediately and continued so actively throughout the period of exposure that the rectal temperature actually rose. Our results are in general agreement with those recently reported by Bazett, Alpers, and Erb (1933) who studied temperature regulation in sub-chronic hypothalamic cats. Although their preparations showed definitely subnormal reactions to heat, they maintained relatively normal body temperatures on exposure to cold. The animals were not subjected to such extremes of environmental temperature as were our cats and dogs and this fact makes it difficult to compare the results of the two sets of experiments. It appears, however, that during survivals of a week or less the hypothalamic cats did not regulate quite as effectively as did our animals of group A and they did not exhibit the excessive response to cold that characterized the latter. In cat 228 and dog 11, animals whose responses were typical of group A, the cerebral ablations were a little less extensive than in the cats of Bazett *et al.* This fact may account for their more normal behavior, but it is also possible to explain the differences between the results of the two series of experiments on the basis of differences in survival times.

Anrep and Hammouda (1932) and Hammouda (1933) studied panting in dogs and discussed its reflex nature. In acute preparations, either under chloralose or a few hours after chloroform-ether anesthesia, Hammouda found that panting occurred normally in response to central stimulation when the neopallium had been removed, but was abolished as effectively by lesions of the "optic thalamus" as by mesencephalic transection. The animals were exposed to slightly higher temperatures than those employed in the present experiments. The results indicated clearly that panting is not a function of the neopallium or corpora striata and that it depends on the diencephalon. On the basis of the published report it is not certain that the "localized injuries to the thalamus," by which Hammouda abolished the capacity to pant, entirely spared the hypothalamus. For this reason his experiments do not conclusively show that it is the thalamus rather than the hypothalamus that is essential for "central" panting. Some light is thrown on this question by our experiments. Although the dogs of group A showed a respiratory response in the warm room, it was greatly delayed, came on only after a distinct rise in rectal temperature and never attained a normal frequency. Since these very striking deficiencies were equally great in dogs 5, 10 and 11 they could be attributed to the absence of the neopallium, but, as already pointed out, the dorsal thalamus, if spared at operation (as in dogs 5 and 10), undergoes a secondary degeneration after removal of the neopallium. Consequently it is possible, as suggested by Hammouda's work, that the deficiencies in the panting response are actually the result of a thalamic defect. In favor of this explanation is the fact that during the excitement stage which immediately followed emergence from the anesthesia of the second cerebral operation dogs 5 and 10 panted in normal fashion. This excitement involved great muscular activity and led to a rise in rectal temperature. A few weeks later, when the secondary degeneration of the thalamus had presumably occurred, panting of the normal type could not be induced by exposure to heat. It is probable that any type of panting (the polypneic or the hyperpneic) that occurs after decortication is dependent on a rise in blood temperature. Hammouda demonstrated that though panting could be induced in acutely decorticate dogs by direct warming of the thalamic region or by warming the carotid blood, it could not be elicited by application of heat peripherally unless the body temperature were raised. He concluded that "reflex" panting is dependent on the cerebral cortex. In this he agreed with Richet (1898) who was the first to make a general study of this subject. Both Hammouda and Sinelnikoff (1929) regard "reflex" panting as a conditioned response.

Keller and Hare (1931) found that animals in which the hypothalamus had been removed and the rest of the brain left intact could not be made to shiver, but exhibited "a remarkable release from control of the heat loss

mechanism," and that "continuous polypnoea and cutaneous vasodilatation supervened." The contrast between this result and those here reported suggests a reciprocal activity between two mechanisms, one for panting, the other for shivering, so that when either is removed the other is released and shows excessive activity. The vasodilatation in both types of preparation, i.e., decorticate and anhypothalamic, indicates that the vasomotor responses are governed by a separate mechanism. Another possible factor operating in the excessive response to cold of the animals of group A is suggested by the hypothesis of Ebbecke (1917), which was modified by Bazett and McGlone (1932), that cold sensations are induced by thermal gradients between the tissues and the blood vessels in the neighborhood of the cold end organs. With the peripheral vasodilatation this gradient should be steeper and consequently the stimulus greater.

The observations recorded here regarding the changes in surface temperature after removal of the motor-sensory area are confirmatory of the results of Eulenburg and Landois (1876). These workers found that stimulation of the cortex of dogs in the region of the cruciate sulcus caused a decrease in temperature of the contralateral feet, whereas ablation of this region of the cortex resulted in a pronounced rise. The latter effect became less distinct in a few days, but was still demonstrable after several weeks or even months. The relatively small changes of surface temperature found in the present experiments are probably due to the delay in making observations until 3 to 4 weeks after the operations. Danielopolu, Radovici and Aslan (1931) also have obtained vasoconstriction of the contralateral arm on electrical stimulation of the cortex in the region of the Rolandic fissure in man. On the other hand, Fulton, Kennard and Watts (1934) found a failure of the reflex vasodilator mechanism in monkeys and chimpanzees after ablation of the premotor area. This same result was mentioned by Kennard (1934) who, in addition, reported that the affected extremities were 2° to 4°F. cooler than the normal. (Kennard is mistaken in stating that "Eulenberg and Landois found that stimulation of the postcentral region in dogs and rabbits resulted in a rise in skin temperature of the contralateral extremities,—." As mentioned above, Eulenburg and Landois obtained a fall in temperature). It seems likely that there is a cortical control of vasoconstrictor and vasodilator mechanisms and that in the dog the vasoconstrictor control is dominant.

It is well known that in temperature regulation several different effector mechanisms come into play. In the present investigation attention was devoted to vasomotor reactions, shivering, hyperpnea, and polypnea. Normal canine panting is a form of polypnea. Sweating is not significant in carnivores. In the chronically decorticate dog overactivity of the shivering mechanism is combined with a profound deficiency of the respiratory response to heat. This fact demonstrates that these two modes of

temperature control do not possess precisely the same cerebral representation. Furthermore there is evidence that both shivering and panting may be induced by either peripheral or central temperature changes. This subject has been investigated by Richet (1898), Sherrington (1924), and Hammouda (1933). The present results are in general accord with the views expressed by these authors. The apparent changes in threshold for panting and shivering that result from decortication suggest more than one channel of excitation for the responses to heat and cold. Whether or not there is a single "center" in the brain responsible for the coördination of the various excitatory and efferent mechanisms cannot be decided on the basis of the data at hand, but one may go so far as to say that several levels of the nervous system, including the cortical, are necessary for normal temperature control.

SUMMARY

The observations reported were made on dogs and cats which survived the removal of portions of the forebrain for long periods (6 weeks to 28 months).

After removal of the neopallium and part of the hippocampus, including the fornix, with subsequent secondary degeneration of large portions of the dorsal thalamus, the dogs showed the following functional changes in their temperature-control mechanisms: 1, chronic vasodilatation, with failure of the skin vessels to constrict normally to cold; 2, absence of true polypneic panting; 3, a very delayed slow type of hyperpnea in response to conditions that promptly induced true panting in the same animals before operation; 4, a rise in rectal temperature before hyperpnea developed; 5, immediate and vigorous shivering in response to cold, frequently producing a rise in body temperature, whereas before operation the same animals showed the usual delayed and less marked reaction with a fall in body temperature.

The cats with equivalent lesions behaved in a similar manner in response to cold. The responses, however, were subject to wider variations than those of the dogs.

There were no differences in temperature control noted between these animals and others with more extensive lesions, which left only the hypothalamus intact, together with the contiguous portions of the medial basal olfactory areas and of the caudal pole of the dorsal thalamus.

All these animals maintained normal body temperatures under ordinary conditions and withstood exposure to heat (37.5° to 41.4°C.) and cold (0.6° to 15.0°C.) for long periods (45 minutes to 4 hours) with relatively small changes.

Observations on animals with unilateral lesions and with ablations of limited portions of the cortex suggest that the cortical control of the vasomotor responses to temperature changes is localized in the contralateral motor-sensory area.

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THE CLEARANCE OF CREATINE IN DOG AND MAN

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Creatine, though normally a constituent of the urine of fish (Denis, 1913–14; Marshall and Grafflin, 1928; Grollman, 1929; Smith, 1929), of amphibia (Smith, unpublished data), of reptiles (Lewis, 1918; Fuse, 1925), and of birds (Thompson, 1916; Hunter, 1928), is generally recognized to be an infrequent constituent of the urine of adult mammals. No studies have been made to determine the mechanism by which the mammalian kidney excretes excess creatine, or retains that which normally circulates in the blood, and the present paper deals with the excretion of creatine at various plasma levels in both dog and man.

In the experiments reported here the excretion of creatinine and xylose were simultaneously observed, clearances of both substances having been suggested as equalling the glomerular clearance (Rehberg, 1926; Jolliffe, Shannon and Smith, 1932, respectively). In those experiments in which the plasma creatine is low, creatinine has been omitted for technical reasons. A few comparisons have been made at high plasma creatine concentrations in the absence of creatinine to determine whether the presence of creatinine affects the excretion of creatine.

For the most part female dogs maintained on a basal diet were used. Urine was collected by catheterization and mid-period bloods obtained from the external jugular vein. The male dog (dog 22) was prepared by explanting the ureteral trigone into the abdominal wall. At the time of experiment 130 (one week following the operation) recovery was incomplete, while by the time of experiment 133 complete healing had been effected. During the experiment the dog stood erect in a harness. Blood was drawn from the external jugular vein at the beginning and end of each three urine collection periods, blood curves drawn and the mid-period values interpolated. Urine was collected by a funnel constructed of rubber dental dam strapped loosely about the body. Xylose and water were administered by mouth, and xylose, sucrose, creatine, and creatinine were given subcutaneously. Heparin was used as an anticoagulant, the bloods centrifuged rapidly and plasma and urine filtrates were prepared immediately. Chemical methods are given in the appendix. All analyses

TABLE 1

Comparison of creatine, creatinine and xylose clearances in the dog at varying plasma levels of creatine

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	PLASMA			CLEARANCE			CREATINE / XYLOSE	CREATININE / XYLOSE
			Xylose	Creatine	Creatinine	Xylose	Creatine	Creatinine		
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*		
Dog 10♀										
	16	5.00	143.0	14.8		26.1	27.6		1.06	
	31	4.40	157.0	13.9		25.1	26.5		1.06	
	46	2.00	154.0	12.3		23.3	24.4		1.05	
	(135)									
	150	3.60	132.0	128.0	20.0	29.6	35.2	38.4	1.19	1.30
107	165	3.57	120.0	110.0	17.0	29.5	35.4	37.2	1.20	1.26
	180	3.30	110.0	97.0	15.1	26.8	33.1	35.1	1.23	1.32
	(255)									
	271	4.50	110.0	49.8	9.25	27.2	31.8	36.0	1.17	1.33
	287	4.13	111.0	45.0	8.86	28.1	33.3	36.6	1.19	1.31
	302	3.57	121.0	41.5	8.42	26.4	31.6	34.3	1.20	1.30
Dog 20♀										
	17	1.30	48.4	3.62		81.6	70.4		0.86	
	32	1.47	49.8	3.21		85.6	68.7		0.80	
	46	1.43	49.3	2.71		83.2	62.3		0.75	
	(135)									
	149	3.14	79.6	64.9		70.8	85.4		1.21	
112	164	3.87	70.1	50.2		81.7	96.0		1.18	
	179	3.67	57.6	36.7		78.0	98.5		1.26	
	(240)									
	255	2.33	49.8	16.6		69.9	92.9		1.33	
	270	2.13	50.8	16.5		61.2	76.9		1.26	
	286	2.31	50.6	15.6		63.2	77.1		1.22	
Dog 22♂										
	(3)									
	5	6.10	59.5	12.4		63.6	73.3		1.15	
	7	6.10	59.8	12.4		63.2	72.8		1.15	
	9	6.10	60.0	12.4		66.8	76.7		1.15	
	(138)									
	141	3.48	79.3	80.4	24.0	60.6	69.5	75.7	1.15	1.25
130	144	3.48	76.0	76.4	23.4	59.8	69.5	75.1	1.16	1.25
	147	3.48	72.8	72.5	22.8	58.6	68.6	73.7	1.17	1.26
	(214)									
	217	1.87	62.5	37.0	17.9	38.8	51.8	58.7	1.33	1.51
	220	1.87	62.2	36.5	17.8	39.8	53.5	58.8	1.34	1.48
	223	1.87	62.0	35.9	17.7	41.0	52.1	59.0	1.27	1.44

TABLE 1—*Concluded*

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	PLASMA			CLEARANCE			CREATINE / XYLOSE	CREATININE / XYLOSE
			Xylose	Creatine	Creatinine	Xylose	Creatine	Creatinine		
Dog 22♂										
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*		
	(3)									
	6	1.86	139.0	58.2	29.4	33.4	40.6	48.7	1.22	1.46
	9	1.86	139.0	57.7	29.2	32.9	40.1	47.3	1.22	1.44
	12	1.86	139.0	57.2	28.9	33.6	41.5	47.6	1.23	1.42
	(16)									
135	19	1.72	139.0	55.7	28.2	32.2	41.7	51.5	1.25	1.55
	22	1.72	139.0	54.8	28.0	35.3	41.9	50.8	1.19	1.44
	25	1.72	139.0	54.0	27.7	36.4	42.2	50.4	1.16	1.38
	(30)									
	33	1.66	137.0	50.8	26.9	35.5	44.5	53.4	1.25	1.50
	36	1.66	135.0	49.3	26.6	37.7	45.1	52.4	1.20	1.39
	39	1.66	134.0	47.8	26.3	37.4	44.6	51.0	1.99	1.36

were performed at least in duplicate and values reported are in all cases averages of the analyses performed without arbitrary corrections.

RESULTS. Typical experiments are reported in table 1 comparing the clearances of creatine, creatinine, and xylose in the dog, and in figure 1 all our results are plotted in terms of the ratio of creatine and creatinine clearance to xylose clearance.

Since renal activity varies so much in different dogs and in the same dog at different times, it is necessary, in studying the relation of creatine clearance to plasma creatine concentration, to refer the observed creatine clearance to some arbitrary standard. Obviously any clearance standard is satisfactory so long as plasma concentration does not affect the clearance. This latter requirement is apparently fulfilled in the dog by both creatinine and xylose (Jolliffe, Shannon and Smith, 1932; Shannon, Jolliffe and Smith, 1932). Since xylose is universally present in our experiments the xylose clearance has been used as the standard of reference.

Figure 1 shows that at low plasma levels of creatine, the creatine clearance approaches zero (i.e., the urine is essentially creatine-free), but that as the concentration of creatine in the plasma is increased, the creatine clearance rises sharply to a level intermediate between the simultaneously determined xylose and creatinine clearances. At plasma creatine concentrations between 15 and 120 mgm. per cent the creatine clearance is invariably higher than the xylose clearance (by an average of 20 per cent) and lower than the creatinine clearance (by an average of 13 per cent).

Within the range of 30 to 120 mgm. per cent the creatine clearance appears to be essentially independent of plasma concentration. There seems to be no evidence that the presence of creatinine in any way affects the excretion of creatine. Whether the converse is true or not (that creatine affects the excretion of creatinine) has not been determined.

A comparison of experiments 107 and 112 with 130 and 133 indicates that there is no perceptible difference in the manner in which creatine is excreted by the male or female dog, a point of interest in view of the greater incidence of creatinuria in women than in men. Whether this similarity obtains at low plasma levels has not been determined because analytical errors at low plasma levels make the point a difficult one to

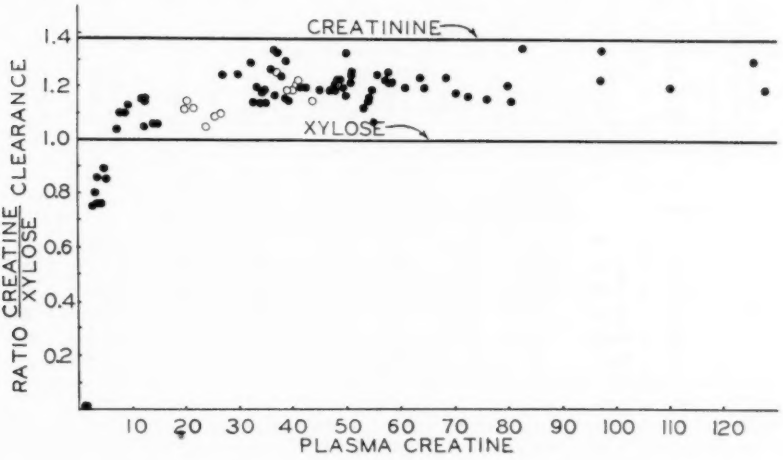

Fig. 1

examine at present. It may be noted that results obtained on both rising and falling plasma creatine curves have been included in figure 1; if there is a difference in the way the kidney handles creatine under the two conditions it is obscured by variations in creatine clearance of unknown origin.

Simultaneous clearances of creatine, creatinine and sucrose are given in table 2. The creatine:sucrose ratios obtained from these data are represented in figure 1 as hollow circles. The relative values of these clearances are the same as when xylose is used as a standard of reference, a result to be expected from the identity of the xylose and sucrose clearances reported by Jolliffe, Shannon and Smith (1932) and Pitts (1933).

It has been shown (Pitts, in press) that glycin administered intravenously causes immediate increase in the urea and xylose clearances, a result which has been interpreted as due to increased glomerular activity.

Simultaneous clearances of creatine, creatinine and xylose before and after the administration of glycin are given in table 3. Glycin causes all three clearances to rise, and on an average by nearly the same absolute amount, with the result that the ratios of creatine and creatinine clearances to xylose clearance fall. Why this occurs is not known but the fact is significant in showing that, whatever the cause of the difference between the xylose, creatine, and creatinine clearances may be, these differences can be altered rapidly and by a physiological change in renal function. The intravenous administration of 50 cc. of 0.5 molar sodium phosphate (pH

TABLE 2

Comparison of sucrose, creatine and creatinine clearances in the dog

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	PLASMA			CLEARANCE			CREATINE / SUCROSE	CREATININE / SUCROSE
			Sucrose	Creatine	Creatinine	Sucrose	Creatine	Creatinine		
						Dog 12 ♀				
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*		
103	11	1.31	109.0	26.5	23.4	28.1	30.8	37.3	1.10	1.33
	21	1.43	107.0	25.4	22.9	23.4	25.6	32.8	1.09	1.40
	31	1.55	103.0	23.8	22.2	24.3	25.4	34.7	1.05	1.43
	41	1.25	99.5	21.4	21.7	24.3	27.3	34.0	1.12	1.40
	52	0.73	95.0	20.1	21.1	20.5	23.6	28.7	1.15	1.40
	64	0.63	90.0	19.8	20.2	20.7	23.2	29.4	1.12	1.42
						Dog 17 ♀				
105	21	1.98	114.0	36.6	16.4	14.1	17.8	21.3	1.26	1.51
	36	1.40	133.0	40.0	18.1	14.7	17.5	21.6	1.19	1.47
	52	0.90	141.0	40.3	18.9	13.7	16.4	20.1	1.20	1.47
	67	0.74	144.0	43.5	19.8	14.4	16.6	20.1	1.15	1.40
	82	0.65	144.0	40.7	19.9	14.4	17.7	21.0	1.23	1.46
	102	0.59	141.0	38.8	20.0	14.1	16.8	20.0	1.19	1.42

7.4) had no effect on either the creatine, creatinine, or xylose clearance (these data are not included here).

A series of five experiments was performed on man, three on the author and two on a volunteer female patient (M. B.) from the Third Medical Division of Bellevue Hospital. From 15 to 70 grams of creatine, 70 grams of xylose, and 10 or 15 grams of creatinine were taken by mouth. In experiments 127, 131, and 132 the creatinine was taken in orange juice and the xylose and creatine in oatmeal. In experiments 128 and 129 these substances were taken in water by stomach tube. Diarrhea, nausea and vomiting attended the three experiments on the author, while there were

no adverse symptoms whatever on M. B. These facts account perhaps for the irregular blood curves in the former and the smoother curves in the latter. Bloods were drawn in the middle of the urine collection period from the antecubital vein. Urine was collected by voluntary voiding in the experiments on the author and by catheterization in the case of M. B. The results of these experiments are reported in table 4.

TABLE 3

The effect of glycin on the creatine, xylose and creatinine clearances in the dog

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	PLASMA			CLEARANCE			$\frac{\text{CREATINE}}{\text{XYLOSE}}$	$\frac{\text{CREATININE}}{\text{XYLOSE}}$
			Xylose	Creatine	Creatinine	Xylose	Creatine	Creatinine		
Dog 20 ♀										
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*		
	15	1.83	143.0	37.8	19.3	28.2	35.0	38.4	1.24	1.36
	30	2.00	141.0	34.4	18.5	32.4	38.1	41.9	1.18	1.29
123	40			5 grams glycin intravenously						
	(45)									
	60	2.77	100.0	26.1	15.4	47.7	53.9	54.5	1.13	1.14
	75	2.03	81.5	23.2	14.1	46.8	53.2	55.0	1.14	1.18
	92	1.30	66.5	19.2	12.6	39.4	47.4	51.0	1.20	1.29
Dog 21 ♀										
	15	4.32	144.0	34.8	16.4	22.7	27.1	29.2	1.19	1.28
	30	3.31	146.0	39.4	18.3	22.7	26.2	29.0	1.15	1.28
	32			5 grams glycin subcutaneously						
126	40			5 grams glycin intravenously						
	(45)									
	60	3.45	124.0	48.8	21.1	32.6	37.2	37.3	1.14	1.14
	75	2.60	98.0	48.5	20.8	34.0	39.6	39.5	1.16	1.16
	90	2.40	80.0	48.6	20.3	35.0	40.6	43.0	1.16	1.23

At plasma creatine levels from 6 to 40 mgm. per cent the average creatine and xylose clearances are identical (1.00 and 1.00 respectively) while the creatinine clearance is higher than the xylose clearance by an average of 49 per cent. As in the dog, there is apparently no sex difference in the excretion of creatine at the plasma levels investigated, and it may be presumed that the reabsorption of creatine in man becomes more or less complete at normal plasma levels.

Discussion. Interpretation of these experiments is obviously dependent upon some knowledge of the amount of creatine filtered at the

glomerulus. If the creatine clearance is greater than the glomerular clearance, tubular secretion must be invoked; if less than the glomerular

TABLE 4

Comparison of creatine, xylose and creatinine clearances in man at varying plasma levels of creatine

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	PLASMA			CLEARANCE			CREATINE / XYLOSE	CREATININE / XYLOSE
			Xylose	Creatine	Creatinine	Xylose	Creatine	Creatinine		
			R. F. P. ♂							
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*		
129	20	5.10	40.0	13.10		92.0	95.0		1.03	
	45	4.65	31.2	9.79		92.4	95.0		1.07	
	66	5.70	26.4	8.12		94.1	98.2		1.04	
	89	3.91	21.3	6.11		91.8	97.8		1.06	
			R. F. P. ♂							
127	15	6.34	28.7	26.40	9.22	106.0	108.0	171	1.02	1.61
	40	3.95	24.7	24.10	8.21	114.0	106.0	172	0.93	1.51
	60	3.88	25.2	23.10	9.09	111.0	110.0	164	0.99	1.48
	80	3.70	29.5	30.00	10.00	103.0	100.0	161	0.97	1.56
			R. F. P. ♂							
128	20	2.90	61.6	43.30	9.70	82.6	80.6	134	0.98	1.62
	40	2.80	51.9	37.60	8.38	88.5	91.2	139	1.03	1.57
	60	2.60	50.4	37.10	8.96	82.2	84.5	128	1.03	1.56
	80	3.00	49.4	41.50	9.23	90.2	91.5	143	1.01	1.59
			M. B. ♀							
131	10	3.43	50.2	21.9	8.62	106.0	103.0	144	0.98	1.38
	21	3.43	54.0	23.4	8.51	95.9	90.3	134	0.94	1.40
	31	3.80	54.9	24.3	8.35	111.0	107.0	152	0.96	1.37
	41	3.50	56.5	25.6	8.31	109.0	108.0	145	0.99	1.33
			M. B. ♀							
132	10	7.30	58.4	20.1	10.8	100.0	97.7	145	0.98	1.45
	21	7.15	60.4	20.7	10.5	92.3	91.2	130	0.99	1.41
	31	7.62	62.6	22.0	10.2	93.3	93.2	131	1.00	1.40
	43	7.92	62.0	22.9	9.67	93.1	93.3	133	1.00	1.43

clearance, it may be surmised that excretion is by filtration alone and that a moiety is reabsorbed in the passage of the filtrate through the tubules.

If the creatinine clearance is a measure of glomerular filtration as suggested by Rehberg (1926) it is evident that at high plasma levels creatine is reabsorbed to an average extent of 13 per cent in the dog and 33 per cent in man.

On the other hand if, in the dog, the xylose clearance more closely approximates the glomerular clear nce as maintained by Jolliffe, Shannon and Smith (1932), creatine at high plasma levels must be secreted in addition to being filtered, the secreted moiety amounting to about 20 per cent of the amount filtered. And if the xylose clearance in man is a measure of the glomerular clearance as maintained by Chassis, Jolliffe and Smith (1933), then at high plasma levels of creatine filtration alone is adequate to account for all the creatine excreted (providing of course that no reabsorption occurs).

Whichever interpretation is accepted, there remains a marked difference between the relative magnitude of the creatine, creatinine and xylose clearances in dog and man that cannot be explained at the present time.

SUMMARY

The clearance of creatine in the dog is a curvilinear function of plasma concentration. At low plasma concentrations the creatine clearance approaches zero, i.e., the urine is essentially creatine-free, but as the plasma concentration is raised the clearance rises sharply. At plasma concentrations between 15 and 120 mgm. per cent the creatine clearance in the dog is intermediate between the simultaneously determined xylose (or sucrose) and creatinine clearances, averaging 20 per cent above the former and 13 per cent below the latter.

Between 6 and 40 mgm. per cent of plasma creatine, the creatine clearance in man is equal to the xylose clearance (the average is identical) while the creatinine clearance averages 49 per cent higher.

No apparent differences were found between the sexes in the mechanism of creatine excretion at the plasma levels of creatine investigated.

The excretion of creatine (either in terms of absolute clearance or relative to xylose) is unaffected by the intravenous administration of neutral sodium phosphate. The intravenous administration of glycin, which increases all renal clearances, raises the xylose clearance relatively more than the creatine clearance.

APPENDIX

METHODS. The analyses of plasma and urine for xylose, sucrose, glucose and creatinine were carried out as described by Jolliffe, Shannon and Smith (1932) and by Shannon, Jolliffe and Smith (1932) in experiments 100 to 117. In the later experiments iron filtrates of both plasma and urine were substituted for the copper filtrate (sugars) and for the tungstic acid filtrate (creatine and creatinine) previously used. The iron filtrate permits identical treatment of blood and urine and analysis for the

sugars, creatine, and creatinine on the same filtrate. All analyses were performed in duplicate or triplicate and results reported are in all cases averages of these analyses.

Creatine was determined by a modification of the Folin method. Two to 5 cc. of a 1:5 or 1:10 plasma or urine filtrate were pipetted into a test tube graduated at 10 cc. Water to 5 cc. was added from a burette and then 1 cc. of 3 N HCl. The tubes were covered with loosely fitting glass bulbs to prevent evaporation of water and HCl and heated in a boiling water bath for four hours, then cooled, and 1 cc. of 3 N NaOH was added. The sides of the tubes were rinsed down and the total volume made to 10 cc. The determination was thereafter identical to that for creatinine. Creatine was thus determined as the difference between creatinine chromogenic power before and after hydrolysis. Preformed creatinine chromogenic substance was determined in all samples but is reported only if in excess of 5 mgm. per cent. Lower values than these probably result in erroneous clearances owing to the presence of non-creatinine substances in blood giving the Jaffé reaction.

Pure solutions of creatinine subjected to the same procedure lost 8 per cent of their chromogenic power, a loss which was independent of the creatinine concentration. This loss of chromogenic power occurred upon adding the acid and alkali without heating or upon the addition of an equivalent amount of NaCl, and was shown to be proportional to the salt concentration. Recoveries of creatine in pure solution are therefore about 8 per cent too low. If appropriate corrections are applied, very satisfactory recoveries of known amounts of creatine and creatinine in blood and urine are possible. Numerous recoveries of from 2 to 100 mgm. per 100 cc. of creatine were performed with an average error of less than 2 per cent. A more satisfactory method of checking the validity of the analytical procedures was to add to blood and urine equal amounts of creatine, creatinine and xylose and to determine the U/P ratios analytically. The following are typical of results obtained in this manner:

RECOVERED FROM PLASMA			$\frac{\text{OBSERVED U/P}}{\text{TRUE U/P}}$		
Creatine	Creatinine	Xylose	Creatine	Creatinine	Xylose
71.2	19.3	75.9	1.02	1.00	1.02
36.6	19.3	75.0	0.97	1.00	1.01
12.5	19.7	75.1	1.02	0.99	1.00
7.52		50.2	0.99		1.00
3.78		50.0	0.98		1.00
1.65		50.0	0.99		1.00

Equally good recoveries of creatine and creatinine were obtained on the tungstic acid filtrates and on the iron filtrates. Attempts to use methods involving hydrolysis of creatine in picric acid were attended with erratic results and were discontinued. Creatine concentrations are expressed in creatinine equivalents without correction for water lost in the hydrolytic change, and no correction is applied for the loss of chromogenic power resulting from the salt present. Both these corrections would be applied equally to blood and urine and hence would be without effect upon the clearances.

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THE CLEARANCE OF CREATINE IN THE PHLORIZINIZED DOG

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The clearance of creatine in the dog was shown in a previous paper (Pitts, 1934) to bear a curvilinear relation to the plasma creatine level. The clearance of normal plasma creatine approaches zero under some conditions, i.e., the urine is essentially creatine-free while, following the administration of exogenous creatine, the clearance rises sharply as the plasma level of creatine is increased. At plasma creatine levels from 10 to 120 mgm. per cent the creatine clearance is intermediate between the simultaneously determined creatinine and xylose clearances, being lower than the former and higher than the latter.

Administration of the drug phlorizin has been shown by Shannon, Jolliffe and Smith (1932) and Pitts (1933) to efface the differences between the simultaneously determined xylose, glucose and creatinine clearances in the dog without significantly affecting the absolute magnitude of the xylose clearance or the level of the xylose clearance relative to the urea clearance. These facts were interpreted as indicating that phlorizin blocked the tubular secretion of creatinine, paralyzed the tubular reabsorption of glucose, and at the same time did not significantly affect the level of glomerular activity as indicated by the xylose clearance. It is thus a problem of major interest to investigate the effect of phlorizin on the clearance of creatine at various plasma levels to see whether the differences between the creatine, xylose and creatinine clearances in the normal dog might not be elucidated to some degree. To this end comparisons of the clearances of creatine, creatinine, and xylose were made in normal dogs, phlorizin was administered either intravenously or both intravenously and subcutaneously, and immediately thereafter comparisons were made of these clearances and in addition the clearance of glucose. The methods used are described in the previous paper (Pitts, 1934).

Results. The results of ten experiments on six different dogs are presented in table 1. In two of these experiments, 121 and 124, no creatinine was administered, the accuracy of low creatine determinations being increased in the absence of any appreciable creatinine in the plasma,

TABLE 1

Comparison of creatine, xylose, glucose, and creatinine clearances under phlorizin at varying plasma levels of creatine

Experiment number	Total concurrent time	Urine flow per minute	Plasma				Clearance				Creatine / Xylose	Creatinine / Xylose	Glucose / Xylose
			Xylose	Creatine	Creatinine	Glucose	Xylose	Creatine	Creatinine	Glucose			
Dog 10 ♀													
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*			
110	15	1.77	174.0	68.2	20.1		26.4	32.8	35.7		1.24	1.35	
	30	1.67	168.0	63.5	18.7		26.8	33.1	37.3		1.24	1.39	
	33			200 mgm. phlorizin/kgm. intravenously									
	(45)												
	60	2.70	136.0	55.0	17.7	132.0	27.4	26.9	27.8	26.9	0.98	1.01	0.98
	75	2.07	120.0	48.3	16.4	111.0	28.5	28.9	31.3	30.3	1.01	1.10	1.06
	90	1.67	108.0	45.6	15.6	109.0	28.1	28.4	31.5	28.6	1.01	1.12	1.02
Dog 10 ♀													
120	15	1.30	51.0	38.6	11.6		48.2	56.0	62.1		1.16	1.29	
	30	1.23	49.8	36.5	11.5		42.5	49.6	55.4		1.17	1.30	
	35			100 mgm. phlorizin/kgm. intravenously									
	(60)												
	75	1.90	40.0	29.3	10.3	98.8	53.4	50.8	53.0	55.6	0.95	0.99	1.04
	90	1.50	35.8	27.0	10.0	93.2	50.0	46.3	49.2	53.0	0.93	0.98	1.06
	105	1.27	33.1	23.4	9.2	90.4	46.6	42.4	46.0	49.1	0.91	0.99	1.05
Dog 18 ♀													
113	17	1.14	145.0	64.2	18.2		27.3	32.7	36.1		1.20	1.32	
	34	1.09	134.0	60.6	18.2		27.0	32.3	35.6		1.20	1.32	
	36			100 mgm. phlorizin/kgm. subcutaneously									
	40			100 mgm. phlorizin/kgm. intravenously									
	(50)												
	70	1.07	100.0	51.5	16.6	80.0	22.1	19.7	23.3	24.1	0.89	1.05	1.09
	85	1.00	94.5	47.6	16.4	82.0	23.4	22.0	25.1	25.0	0.94	1.07	1.07
	106	0.91	86.0	44.3	16.0	84.5	22.6	21.0	24.3	24.6	0.93	1.07	1.09
Dog 18 ♀													
117	0			100 mgm. phlorizin/kgm. subcutaneously									
	15			100 mgm. phlorizin/kgm. intravenously									
	(30)												
	45	1.81	80.7	56.0	24.9	102.0	31.2	28.5	31.9	32.2	0.91	1.02	1.03
	60	1.52	83.3	57.8	26.1	94.0	29.6	27.1	30.4	31.2	0.92	1.03	1.05
	80	1.37	80.4	54.3	25.3	93.0	29.2	27.0	30.6	30.1	0.93	1.05	1.03
	96	1.25	72.9	48.2	24.3	95.0	29.5	27.0	30.5	31.6	0.92	1.03	1.07
Dog 12 ♀													
115	15	1.73	154.0	54.9	24.0		25.3	27.1	33.2		1.07	1.31	
	30	1.32	145.0	53.0	24.2		20.2	22.6	26.7		1.12	1.32	
	37			100 mgm. phlorizin/kgm. intravenously									
	(45)												
	60	1.37	124.0	51.9	24.0	78.0	19.8	18.1	20.6	18.7	0.92	1.04	0.95
	75	1.33	116.0	48.1	23.7	74.0	20.8	19.6	21.9	21.2	0.94	1.05	1.02
	90	1.27	107.0	46.0	22.9	66.5	21.8	20.2	23.3	22.9	0.93	1.07	1.05

TABLE 1—*Concluded*

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	PLASMA				CLEARANCE				CREATINE / XYLOSE	CREATININE / XYLOSE	GLUCOSE / XYLOSE
			Xylose	Creatine	Creatinine	Glucose	Xylose	Creatine	Creatinine	Glucose			
			Dog 12♀										
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*			
116	0		200 mgm. phlorizin/kgm. subcutaneously										
	25		200 mgm. phlorizin/kgm. intravenously										
	(45)												
	60	1.20	108.0	66.0	30.6	97.0	14.8	14.5	15.0	14.8	0.98	1.01	1.00
	75	1.03	104.0	63.5	30.2	94.0	13.6	12.9	14.0	13.2	0.95	1.03	0.97
	90	1.10	100.0	61.2	29.7	89.0	15.2	14.2	15.6	15.1	0.93	1.03	0.99
	105	1.03	95.8	57.6	29.0	87.0	14.8	14.1	15.3	14.8	0.95	1.03	1.00
			Dog 13♀										
121	16	1.00	169.0	5.27			9.20	7.85			0.85		
	30	1.03	169.0	4.82			9.06	8.09			0.89		
	37		200 mgm. phlorizin/kgm. intravenously										
	(45)												
	60	1.05	139.0	3.87		130.0	9.29	6.27		9.10	0.68		0.98
	76	0.78	126.0	3.50		115.0	8.89	5.40		9.10	0.60		1.02
	92	0.63	109.0	3.16		106.0	8.28	4.35		8.28	0.53		1.00
			Dog 20♀										
124	15	5.50	162.0	4.46			30.9	23.4			0.76		
	30	5.90	159.0	3.89			30.9	23.5			0.76		
	35		200 mgm. phlorizin/kgm. intravenously										
	(45)												
	60	2.70	125.0	2.58		119.0	31.6	23.2		33.1	0.73		1.05
	75	2.37	112.0	2.25		106.0	33.9	24.0		34.9	0.71		1.03
	90	1.97	101.0	2.10		95.0	34.5	22.4		37.9	0.65		1.10
			Dog 20♀										
125	0		200 mgm. phlorizin/kgm. intravenously										
	(20)												
	35	6.73	59.6	130.0	22.1	110.0	41.6	39.2	42.3	42.3	0.94	1.02	1.02
	50	5.66	55.5	115.0	21.7	109.0	39.5	36.2	38.4	36.6	0.92	0.97	0.93
	65	4.26	51.8	92.0	20.5	108.0	33.8	34.5	36.2	36.8	1.02	1.07	1.09
	80	3.07	47.0	82.0	19.0	106.0	30.9	30.4	33.6	34.6	0.98	1.09	1.12
			Dog 22♂										
134	(2)												
	4	5.60	87.8	48.8	29.7		50.2	61.7	70.3		1.23	1.40	
	6	4.00	87.3	48.2	29.6		45.5	56.0	62.8		1.23	1.38	
	8	3.10	86.9	47.7	29.5		49.0	59.2	66.8		1.21	1.36	
	18		200 mgm. phlorizin/kgm. intravenously										
	(30)												
	33	2.60	72.7	41.9	27.2	106.0	48.8	47.1	48.1	48.6	0.97	0.99	1.00
	36	2.53	70.8	40.5	26.9	101.0	49.8	48.0	49.7	50.0	0.96	1.00	1.00
	39	2.50	69.0	39.0	26.7	96.0	51.8	48.9	51.2	50.0	0.95	0.99	0.97

and in experiments 116, 117 and 125 no periods were taken before phlorizin since the dogs had a short time previously received phlorizin and it was felt that some effect of the drug might be carried over the few intervening days.

An examination of the data in table 1 reveals that differences existing in the normal dog between the creatine, xylose, creatinine and glucose clearances are not found in the phlorizinized dog. Instead, the average clearances of these four substances (except for creatine at low plasma levels) fall within a range of ± 5 per cent. Graphically the data in figure 1 indicate that a curvilinear relation exists in the phlorizinized as in the normal dog (Pitts, 1934) between creatine clearance and plasma creatine. In contradistinction to the normal dog, however, the asymptote approached in the phlorizinized dog is not intermediate between the xylose and cre-

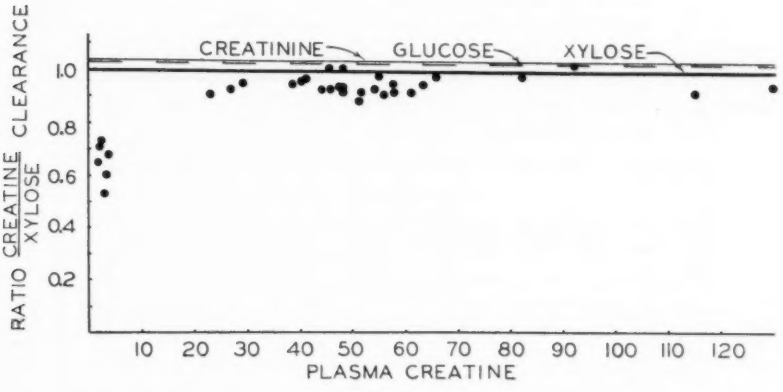

Fig. 1

atinine clearances, which are in turn widely separated, but is within a few per cent of the almost identical glucose, xylose and creatinine clearances. The clearance of creatine at low plasma levels in the phlorizinized dog as in the normal dog is definitely below the xylose clearance. Whether this relation has been significantly affected by phlorizin can scarcely be determined since the clearance is changing so rapidly for each increment of plasma concentration within this range. Whether the differences between the average clearances in the phlorizinized dog are significant is not a point we wish to argue at present (when related to the xylose clearance these average, creatinine 1.03, glucose 1.03, and creatine 0.95). But the change in these ratios from creatinine 1.34 and creatine 1.19 before phlorizin to the above mentioned values after phlorizin is certainly significant.

Discussion. The close correspondence in the phlorizinized dog between the creatine, xylose, glucose and creatinine clearances may logically mean

one of several things; *a*, that the glomerular level of activity is within or close to the range of these clearances; *b*, that these clearances represent some physiologically constant fraction of the glomerular clearance; *c*, that the phlorizinized tubule is damaged to such a degree that practically all differential impermeability is lost and thus these clearances are some physiologically inconstant fraction of the glomerular clearance, which is higher by an amount dependent on the extent of tubular damage.

In figure 2 are presented average clearances on four dogs obtained in 5 experiments in which the periods following phlorizinization followed im-

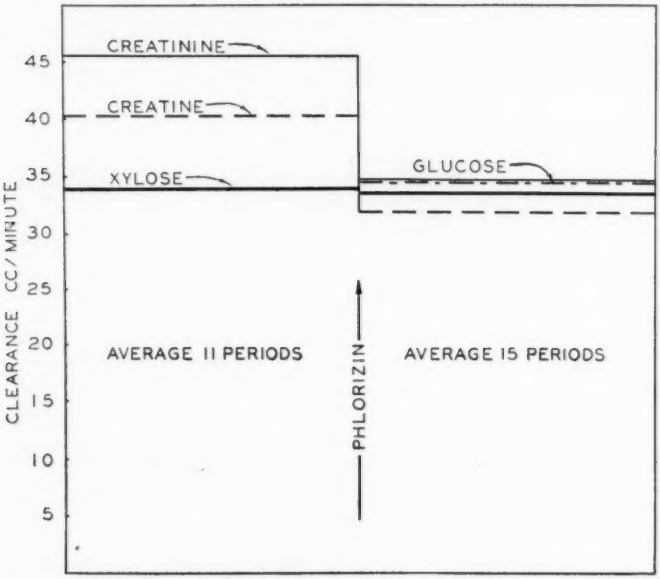

Fig. 2

mediately after the normal control periods. In order to avoid any weighting of the averages by experiments in which the absolute clearances are high all clearances were corrected to what they would have been had the xylose clearance before phlorizin been the average value of 34.0 cc. per minute. As a matter of fact this correction causes no appreciable change in the results. The experiments averaged are those in which creatine, creatinine, xylose and glucose clearances were determined before and after phlorizin, namely, experiments 110, 113, 115, 120, 134. It may be seen by inspection of figure 2 that the creatinine clearance dropped from an average value of 45.5 cc. per minute before phlorizin to 34.8 cc. after

phlorizin, the creatine clearance dropped from 40.3 cc. to 31.9 cc., while the xylose clearance remained practically constant at 34.0 cc. and 33.6 cc. The glucose clearance rose from some undetermined but low value to 34.6 cc. By inspection of the figures in table 1 it may be seen that averaging the data has not obscured any significant trend; rather it emphasizes the constant drop in the creatine and creatinine clearances and the relatively insignificant variations in the xylose clearance.

The constancy of the xylose clearance before and after phlorizin in these experiments argues against marked tubular damage and a loss of differential impermeability as accounting for the correspondence between the xylose, creatine, creatinine, and glucose clearances under phlorizin. No evidence is at present at hand either for or against the thesis that these clearances represent some physiologically constant fraction of the glomerular clearance.

The most logical assumption to make is that the glomerular clearance is within the range of these clearances and that in the phlorizinized dog creatine as well as creatinine, glucose and xylose are eliminated by filtration at the glomerulus alone, that little tubular reabsorption occurs, and that any one of these clearances may, in the phlorizinized dog, be used to measure the glomerular clearance with an accuracy but little less than the accuracy of the analytical method itself.

But the question of the clearance of creatine, creatinine, and xylose in the normal dog is a less certain matter. If the creatinine clearance in the normal dog is a measure of glomerular clearance as maintained by Rehberg (1926) then creatine in the normal dog is reabsorbed to the extent of 11 or 12 per cent and xylose to the extent of 25 to 30 per cent. Phlorizin thus scarcely affects the reabsorption of creatine, perhaps blocking 5 or 6 per cent, but blocks all but about 3 per cent of the xylose reabsorbed. According to such an explanation no secretory power of the tubule for any of these substances in the normal dog need be invoked. One would thus expect the creatinine and creatine clearances to remain constant and the xylose clearance to rise to their level after phlorizin. Instead the opposite is true, the xylose clearance remains constant and the creatinine and creatine clearances drop to the xylose level (fig. 2).

Jolliffe, Shannon and Smith (1932) and Shannon, Jolliffe and Smith (1932) emphasized the constancy of the ratio of the xylose and urea clearances before and after phlorizin, and used this point as argument against reabsorption of xylose in the normal dog. White and Monaghan (1933b), on the other hand, suggested that the correspondence of the creatinine and xylose clearances after phlorizin, as observed by the above mentioned investigators was due to a drop in the glomerular clearance as indicated by creatinine equal to the degree to which this drug blocked the

reabsorption of xylose.[1] Thus the constancy of the xylose clearance before and after phlorizin is in this interpretation purely coincidental. If this is true, and accepting Jolliffe, Shannon and Smith's observations on the constancy of the urea:xylose ratio, one is forced to conclude that phlorizin blocks not only the reabsorption of xylose but of urea as well, and to the same extent. Unfortunately we were unable to include urea clearances in our present study.

A new line of evidence obtained in this laboratory which is not at present ready for presentation might be construed to indicate that White and Monaghan's interpretation is correct. If such proves to be the case the rate of glomerular filtration in the normal dog may be above the maximum creatine clearance. For this reason final opinion on the question is reserved.

White and Monaghan (1933b) failed to find consistent identical clearances of creatinine, glucose and xylose after phlorizin as reported by Shannon, Jolliffe and Smith (1932). Special care was therefore used in this investigation to check this point, and as reported above we find that creatinine, glucose, xylose and the newly studied substance creatine give average clearances in the phlorizinized dog agreeing within ± 5 per cent. Although the Folin (1929) sugar method has been used exclusively in the present experiments its application either to copper filtrates or to iron filtrates has yielded the same results. Satisfactory recoveries of creatine, creatinine, xylose and glucose from copper, iron, and tungstic acid filtrates

[1] It is important to note that the chief argument of White and Monaghan (1933b) for the reabsorption of xylose is the reported constancy of the creatinine clearance before and after phlorizin and the rise in xylose clearance after phlorizin. They average all data on dog 1 in experiments with and without phlorizin (although these were performed on different days) and find an increase in xylose clearance from 34.8 cc. to 43 cc., the creatinine clearance remaining constant. However in dog 2 the xylose clearance remains fairly constant at 24.6 and 25.9 cc. and the creatinine drops from 46.8 cc. to 33.1 cc. In the only two experiments in which the phlorizin periods immediately succeeded the normal periods (comparable to our experiments) they find in experiments 25 the xylose clearance decreasing from 42.6 cc. before phlorizin to 39.6 cc. after phlorizin, and the creatinine dropping from 59.6 cc. before to 47.5 cc. after. In experiment 26 the xylose clearance increases from 34.1 cc. to 41.7 cc. and the creatinine clearance remains constant at 55.2 and 55.7 cc. In other words, in half their cases they find no appreciable change in creatinine clearance and in the other half no change in the xylose.

Our experiments, with the exception of experiments 120 and 134, were performed on dogs maintained on a basal diet in which the clearance tends to remain more constant throughout a long experiment. Furthermore our experiments were performed with a minimum of elapsed time between the normal periods and the phlorizin periods. Their experiments on dog 1 without phlorizin on different days show marked variability of clearance, and the danger of comparing experiments on the same dog performed days and weeks apart and ascribing these differences to phlorizin is evident.

have convinced us that the differences between White and Monaghan and ourselves are not due to analytical errors in our methods. White and Monaghan state in one paper (1933a) that the blood in their experiments was "centrifuged immediately and the serum removed and placed in the refrigerator; the protein free filtrates were usually prepared on the day of the experiment"; and in the other paper (1933b) they state "filtrates of both serum and urine were made on the day of the experiment." We may emphasize that in all our experiments the plasma and urine filtrates were prepared immediately after rapid centrifugation of the blood, a matter of some importance in dealing with glucose clearances. No arbitrary corrections have been made on the plasma creatinine for non-creatinine chromogenic substances, as was done by White and Monaghan (1933b). Apart from these points the discrepancies between the two laboratories remain unexplained.

SUMMARY

The creatine clearance in the phlorizinized dog is a curvilinear function of plasma creatine concentration below 5 mgm. per cent. Between 20 and 130 mgm. per cent the creatine clearance is essentially independent of plasma creatine concentration.

The simultaneously determined creatine, creatinine, xylose and glucose clearances agree within an average of ± 5 per cent in the phlorizinized dog, which is presumptive evidence that the clearance of any one of these substances approximates the glomerular clearance.

The creatine and creatinine clearances drop to the xylose level, the glucose clearance rises to the xylose level, and the xylose clearance itself does not appreciably change following the administration of phlorizin.

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THE INFLUENCE OF NEUTRAL SODIUM-SALT SOLUTIONS ON CHEMICAL STIMULATION

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When an isolated frog's muscle is immersed in a pure isotonic Na-salt solution, the most obvious of all the changes that may occur in the muscle is the production of spontaneous rhythmic twitches. These twitches were first observed by Biedermann in 1879 in a curarized muscle placed in a 0.5 per cent NaCl solution made weakly alkaline by Na_2CO_3, and were studied more thoroughly by Biedermann (1880), Ringer (1886), Loeb (1899) and many others. These twitches were also rightly ascribed by the early workers to the direct action of the salt solution on the muscle fibres, for they can be obtained in curarized as well as in uncurarized muscles.

The production of these spontaneous twitches suggests an increase in the irritability of the muscle. Thus, with particular reference to chemical stimulation, Zoethout (1904) mentioned an increase in irritability towards KCl in muscles placed in a NaBr or $NaNO_3$ solution. Lillie (1910) showed that the response of the skeletal muscle to various forms of chemical stimulation is increased by a preliminary immersion in a pure isotonic Na-salt solution, a process which may be designated for convenience as sensitization, and that the order of the relative sensitizing action for isotonic solutions of the following Na-salts is, in general, as follows:

$$NaCl < NaBr < NaNO_3 < NaClO_3 < NaI < NaCNS$$

In the present work a more extensive study of the conditions of the action of these Na-salt solutions on chemical stimulation will be presented.

Frog's sartorii were used throughout in these experiments. Experiments were performed in pairs, using a pair of sartorii from the same frog. Each muscle was attached to a light lever for graphic registration and was immersed in a beaker of about 250 cc. of Ringer's solution, which could be easily and rapidly replaced by another beaker of a sensitizing solution without disturbing the muscle. The procedure of experimentation has consisted in immersing a muscle in a sensitizing solution (e.g., a pure isotonic $NaNO_3$ solution) for a given period of time (the sensitization process); then this sensitizing solution was rapidly replaced by a stimulating solution (stimulation process). The stimulating solution contained 1 volume

of a M/8 KCl solution and 24 volumes of a M/8 NaCl solution (i.e., 5 millimols KCl per liter isotonic NaCl solution). This solution of the KCl and NaCl mixture usually produced a slight contracture in the muscle only, on transfer from Ringer's solution, and its strength of stimulation may be considered as threshold or slightly above. After a period of immersion in a pure isotonic Na-salt solution, the muscle responded immediately to the same stimulation with increased vigor, as shown by the increased height of the contracture (fig. 1). The increase in height before and after the sensitization, then, is a measure of the change in irritability

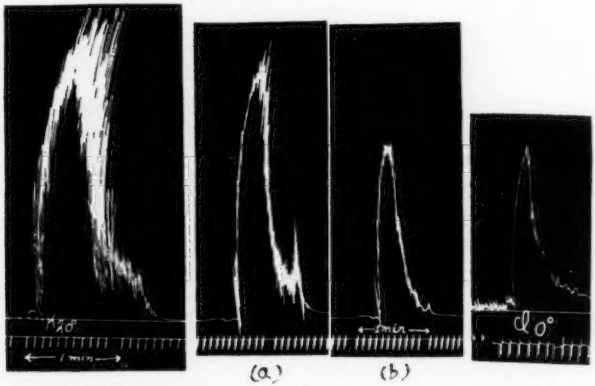

Fig. 1 Fig. 2 Fig. 3

Fig. 1. An example of the increased response of the skeletal muscle to the stimulation of an isotonic NaCl solution containing 5 millimols KCl per liter, after the muscle was immersed in a pure isotonic $NaNO_3$ solution for 6 minutes. The response to the same stimulation before sensitization was negligible. In all the figures (read from left to right) the change of solutions was automatically marked by the rise and fall of the base line; a rise indicates the removal of a solution, and a fall indicates the application of another solution.

Fig. 2. The response of a curarized muscle to the chemical stimulation (a) after 10 seconds and (b) after 5 seconds in a pure isotonic NaCNS solution.

Fig. 3. Effect of changing from a pure isotonic NaCl solution at 25° to another at 0°C. Note the production of contracture in the latter.

to the particular chemical stimulation produced by the sensitizing solution under these conditions; and was employed throughout this work in the evaluation of the results obtained with different sensitizing solutions under different conditions.

The effect of anions on the sensitization. As shown by Lillie (1910) in the frog's gastrocnemius,[1] the relative effectiveness of different Na-salt solu-

[1] The author has confirmed Doctor Lillie's results on gastrocnemius with stimulating solution containing KCl and NaCl.

tions in sensitizing the muscle to various forms of chemical stimulation follows the order of the Hofmeister series for anions. The same relationship is also true for the sartorius. The following experiment on a pair of sartorii from a curarized frog illustrates the influence of the anions very well. In this experiment each sartorius was sensitized in a pure isotonic Na-salt solution for two minutes before it was transferred into the stimulating solution. A period of at least 15 minutes was allowed for recovery in the Ringer's solution between two successive trials. The order of sensitization with a series of sensitizing solutions was reversed for the

TABLE 1

Percentage increase in response of a pair of curarized sartorii to chemical stimulation after a period of sensitization of two minutes in different pure isotonic Na-salt solutions

NaCl	NaBr	$NaNO_3$	$NaClO_3$	NaI	NaCNS
5	30	41	46	63	*100*

TABLE 2

Percentage sensitization for different Na-salt solutions at different concentrations (diluted with Ringer's solution)

VOLUMES PER CENT PURE ISOTONIC Na-SALT SOLUTION	NaCl (2)*	NaBr (4)	$NaNO_3$ (4)	NaI (4)	NaCNS (2)
100	*100*	*100*	*100*	*100*	*100*
80	50	74	73	*110*	106
60	0	27	22	98	108
50			16		
40		7	0	12	94
20				4	25
10					8
5					1.5

* The numbers in the brackets indicate the number of determinations.

two muscles to rule out any possible effect of fatigue or other changes in the tissue. The increase in height of response after the immersion in the NaCNS solution was taken as 100 per cent; the relative increases for the other Na-salt solutions are summarized in table 1.[2]

It is obvious from table 1 that NaCNS is the most effective sensitizing solution; the other solutions follow the order:

$$NaCl < NaBr < NaNO_3 < NaClO_3 < NaI < NaCNS$$

[2] Comparisons between any given two or three Na-salt solutions on many different pairs of sartorii gave the same relative position in the lyotropic series.

The effect of concentration of the Na-salt on the sensitization. The sensitization depends upon the concentration of the pure salt dissolved in the sensitizing solution. This is best shown by mixing pure isotonic Na-salt solutions with different amounts of Ringer's solution.[3] In table 2 the increase in height of contracture in the stimulating solution after a period of sensitization in a given pure Na-salt solution for two minutes was taken as 100 per cent, and the responses after similar sensitizations in solutions of lower concentrations were calculated on the same percentage basis.[4]

When the values of the relative sensitizing action of the pure isotonic Na-salt solutions given in table 1 are substituted into table 2, the percentages of sensitization at other concentrations are recalculated and shown in table 3. Thus a composite graph can be constructed (fig. 6) to

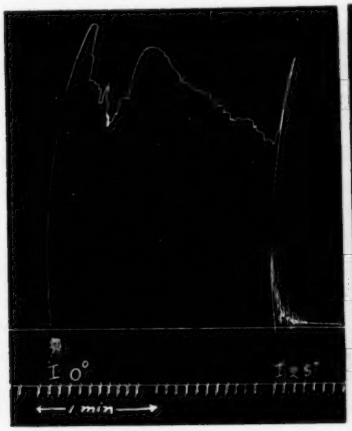

Fig. 4

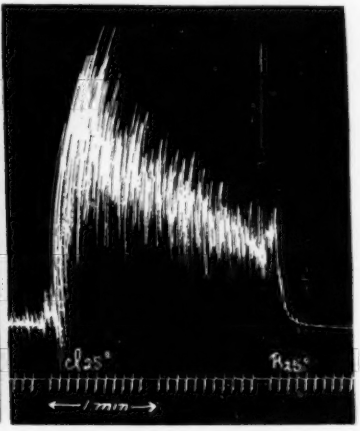

Fig. 5

Fig. 4. Effect of changing from a Ringer's solution at 25° to an isotonic NaI solution at 0°C. Note the immediate response of the muscle. Two minutes later, the cold NaI solution was replaced by another NaI solution at 25°C.

Fig. 5. Effect of changing from an isotonic NaCNS solution to an isotonic NaCl solution; both at 25°C.

[3] Ringer's solution was used instead of some non-electrolyte solutions, ordinarily considered as indifferent, on account of the fact that these solutions (isotonic sucrose and dextrose solutions) were found to possess some undesirable action on the muscle by themselves.

[4] $NaClO_3$ was left out of the series at this point on account of its anomalous behavior in the presence of NaCl. After a muscle is sensitized in a mixture of $NaClO_3$ and NaCl, or $NaClO_3$ and Ringer's solution, and an increased response to the chemical stimulation is obtained, subsequent sensitization in the same solution, or in a pure isotonic $NaClO_3$ solution, will no longer produce the same effect, although the muscle still responds to the action of the other Na-salt solutions.

show the relation between the concentration and the sensitizing action of different solutions.

Effect of duration of sensitization on the increase in response to chemical stimulation. The rapidity of the sensitizing action shows also a specific dependence on the nature of the anions. Thus in a pure NaCNS solution, a considerable increase in response is obtained after a period of sensitization of 10 seconds or less (fig. 2), and the maximal response is reached in less than half a minute. The durations of sensitization necessary to produce the maximal response for the other Na-salt solutions follow also the lyotropic order. Table 4 (fig. 7) gives the relative responses for different durations of sensitization in different solutions. The response after a period of sensitization of 10 minutes was considered as 100 per cent for NaCl; 6 minutes, for NaBr and $NaNO_3$; 2 minutes, for NaI; and 1 minute, for NaCNS. It should be mentioned, however, that the actual maximal response is not the same for all the Na-salt solutions.

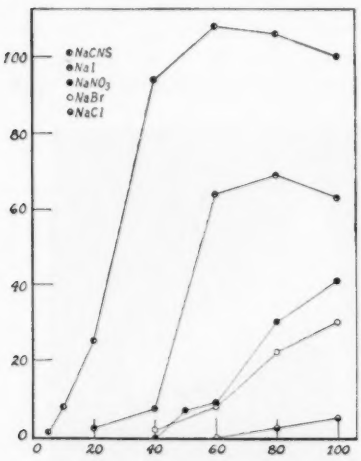

Fig. 6. A composite picture showing the relative sensitization effect and the influence of the concentration of the Na-salt solutions. Ordinates: percentage increase in response. Abscissae: percentage volumes of isotonic Na-salt solution in Ringer's solution.

Effect of $CaCl_2$, $MgCl_2$, $SrCl_2$ and HCl on the sensitizing action of the Na-salt solutions. The action of the pure Na-salt solutions in sensitizing a muscle to chemical stimulation is readily antagonized by the Ca-ion, and the concentration of Ca-salt required for this antagonism increases with the sensitizing effectiveness of the Na-salt. Hence the lyotropic order is again shown in the experiments on antagonism; this is summarized in table 5 (fig. 8).

Similarly, the minimal concentrations of HCl, $MgCl_2$ and $SrCl_2$ necessary for complete suppression of the sensitizing action of several Na-salt solutions were determined, and the results are summarized in table 6.[5]

[5] $BaCl_2$ differs from the other chlorides of the other alkali earth metals in that it possesses a powerful stimulating action, increasing the spontaneous twitches of muscle placed in pure Na-salt solutions (Ringer, 1886; Loeb, 1899). Thus even an addition of 0.01 millimol $BaCl_2$ to a liter of isotonic NaI solution produces much more vigorous twitches and considerable contracture and tends to diminish the response to the chemical stimulation. At higher concentrations of $BaCl_2$, these twitches and contracture obscure any subsequent response to chemical stimulation.

The order of the relative antagonistic action of these substances on sensitization indicated by these experiments is:

$$SrCl_2 < MgCl_2, CaCl_2 < HCl$$

Effect of temperature on the sensitizing action of the Na-salt solutions. In general, low temperature tends to increase the stimulating action as well

TABLE 3

Relation between the concentration of the Na-salt solutions and their relative sensitizing action

VOLUMES PER CENT PURE ISOTONIC Na-SALT SOLUTION	PERCENTAGE INCREASE IN RESPONSE IN				
	NaCl	NaBr	$NaNO_3$	NaI	NaCNS
100	5	30	41	63	*100*
80	2.5	22	30	69	106
60	0	8	9	64	108
50			7		
40		2	0	7.4	94
20				2.5	25
10					8
5					1.5

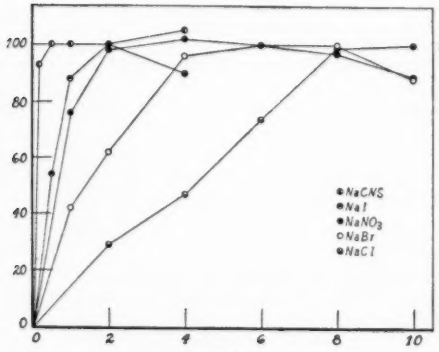

Fig. 7

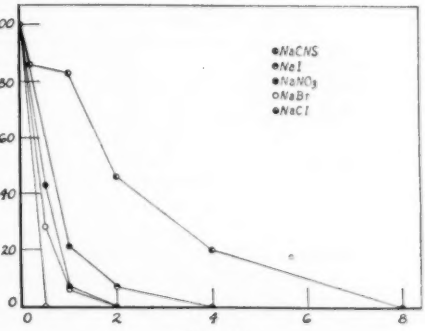

Fig. 8

Fig. 7. Effect of duration of sensitization in the Na-salt solutions. Ordinates: percentage increase in response. Abscissas: time of sensitization in minutes.

Fig. 8. Antagonistic action of $CaCl_2$ on sensitization. Ordinates: percentage increase in response. Abscissas: concentration of $CaCl_2$ in millimols per liter.

as the sensitizing action of the Na-salt solutions. Thus a Na-salt solution may produce more twitches in a muscle at room temperature, but a high contracture at low temperature, as illustrated by figures 3 and 4. The contracture produced in a cold Na-salt solution is not due to a sudden

change in temperature alone (although under certain conditions a sudden decrease in temperature may have a stimulating action by itself), for

TABLE 4

Percentage increase in response to chemical stimulation after immersions for various durations in different pure isotonic Na-salt solutions

	DURATION							
	10 seconds	30 seconds	1 minute	2 minutes	4 minutes	6 minutes	8 minutes	10 minutes
NaCl				29 (6)*	47 (4)	74 (7)	99 (2)	*100*
NaBr			42 (5)	62 (4)	96 (3)	*100*	100 (9)	88 (5)
$NaNO_3$			76 (3)	98 (6)	102 (5)	*100*	97 (4)	89 (4)
NaI		54 (6)	88 (8)	*100*	90 (7)			
NaCNS	93 (7)	100 (6)	*100*	100 (6)	105 (4)			

* The numbers in the parentheses indicate the number of determinations.

TABLE 5

Percentage increase in response (100 per cent in pure Na-salt solutions) to chemical stimulation with addition of $CaCl_2$ to different Na-salt solutions (average of 2 determinations for each figure)

Duration of sensitization was 2 minutes

	CONCENTRATION OF $CaCl_2$ (m.M. PER LITER)						
	0	0.2	0.5	1	2	4	8
NaCl	*100*		0				
NaBr	*100*		28	6	0		
$NaNO_3$	*100*		43	7	0		
NaI	*100*			21	7	0	
NaCNS	*100*	86		83	46	20	0

TABLE 6

Minimal concentrations (millimols per liter) of various substances necessary for complete suppression of the sensitizing action of pure Na-salt solutions

	NaCl	NaBr	$NaNO_3$	NaI	NaCNS
HCl		0.02	0.2		1*
$CaCl_2$	0.5	1–2	1–2	2–4	8
$MgCl_2$		4	4		8
$SrCl_2$			8		16

* M/1000 HCl in an isotonic NaCNS solution produced contracture in the muscle (acid contracture).

considerable contracture occurs even on change from a Ringer's solution at 0°C. to a pure Na-salt solution at the same temperature.

The effect of temperature on the sensitization is illustrated in a series of experiments summarized in table 7.

In the above series of experiments both the sensitizing solutions and the stimulating solution were kept at the same given temperature. With the exception of NaCl, the response is, in general, greater at low temperature than at high temperature. In other words, there is a negative temperature coefficient for the sensitization to chemical stimulation.

TABLE 7

Percentage increase in response of muscle to chemical stimulation at different temperatures

Both the sensitizing solution and the stimulating solution were kept at the same given temperature. The response at 20° was taken as 100 per cent.

SENSITIZING SOLUTION	NUMBER OF DETERMINATIONS	DURATION OF SENSITIZATION IN MINUTES	TEMPERATURE OF SOLUTIONS			
			0°	10°	20°	30°
NaCl	4	6	0	44	*100*	74
NaBr	4	6	400	250	*100*	64
$NaNO_3$	2	6	138	106	*100*	88
NaI	2	4	123	109	*100*	58
NaCNS	2	2	106	94	*100*	74

TABLE 8

Effect of exchange of anions on the spontaneous twitches of the skeletal muscle

FROM	TO				
	NaCl	NaBr	$NaNO_3$	NaI	NaCNS
NaCl		−−	−−?	−−	−−−−−−−−−−
NaBr	++++++?−		−−	−?	−−
$NaNO_3$	++++?−−	−−		−−	−−−−
NaI	++	??	−−		−−−−
NaCNS	+++++++ ++++	++	+++++++ −	++++	

Analysis of the nature of the stimulating solution. The stimulating solution employed in these experiments contains 5 millimols KCl in a liter of an isotonic NaCl solution. The stimulation is really twofold. In the first place, the K-ion has a primary stimulating action followed by inhibition. Gellhorn (1928) has shown that the K-contracture is increased by the action of the Na-salt solutions in the same order as the Hofmeister anion series as described in this work. Secondly, in the case of Na-salts of anions other than chloride, there is a second effect which is apparently referable to an exchange of anions. This effect is illustrated in the experiments summarized in table 8. A sartorius was immersed in a given

pure Na-salt solution for four minutes at room temperature, and then transferred to a pure solution of another Na-salt at the same temperature. Changes in the amount and height of the spontaneous twitches are shown in table 8.

In table 8 each + sign indicates a single determination in which a definite increase in the amount and height of the spontaneous twitches, or even the production of considerable contracture, was the immediate result of change from one solution to another; a − sign, no increase; and a ? mark, a doubtful case. Thus, while change from NaCl to NaBr, $NaNO_3$, NaI and NaCNS did not increase the twitches, change from any of them to NaCl produced a definite and well-marked increase. No particular effect was seen on replacing any salt by NaCNS, yet change from NaCNS to any other salt of the series always produced vigorous twitches accompanied by contracture (fig. 5).[6] The effect of the interchange of solutions between NaBr, $NaNO_3$ and NaI was not clearly defined.

Discussion. The increase in response of the skeletal muscle to chemical stimulation under the conditions described above appears to be related to changes in the physical state of the structural colloids of the plasma membrane of the muscle fibres, as suggested by Lillie (1910). This assumption is justified in view of the following facts.

The order of the relative sensitizing action of the pure Na-salt solutions is that of the lyotropic series. This characteristic order of the relative influence of anions on the physical state of colloidal substances was first demonstrated by Hofmeister (1888) and his student (Lewith, 1888) on the precipitation of proteins. According to the colloidal theory of the action of ions in living tissues (Höber, 1926; Lillie, 1933), these anions have a dispersing action on certain structural colloids of the plasma membrane, and this change in some way facilitates the process of stimulation. The differential action of the anions may be referred to their differential adsorption by the colloidal substance. Thus Rona and Michaelis (1919) have shown that these anions are adsorbed in the same relative order by blood charcoal.

The rapidity with which the muscle responds to the change of its chemical environment indicates that the primary action of the ions is upon the surface of the muscle fibres. Immersion of a muscle in a pure Na-salt solution for one minute or less is sufficient to give rise to a well-marked increase in response. Furthermore, the response to subsequent chemical stimulation appears almost simultaneously with the change of solution. Such rapid response would exclude any possibility of diffusion, or penetra-

[6] Incidentally the indirect CNS-contracture described by Gellhorn (1931) in the frog's sartorius on change from a mixture of Ringer's solution and isotonic NaCNS solution to a pure Ringer's solution is apparently due to a partial exchange of anions.

tion, as the primary cause in the process of stimulation; on the contrary, it suggests strongly some immediate action of the ions on the plasma membrane of the muscle fibres.

The negative temperature coefficient of the sensitization process (the response being greater at low temperature) seems again to rule out the possibility of diffusion as a primary factor in the change of irritability. On the other hand, processes that are characterized by a negative temperature coefficient, such as surface tension and its related phenomena (e.g., adsorption) are known to be important factors in relation to changes of the colloidal state. Finally Mond and Amson (1928) have shown by perfusion experiments that the resting uninjured muscle fibres are impermeable to anions. It is, therefore, reasonable to believe that the primary process involved in the action of these Na-salt solutions is an increase in the dispersion (or perhaps rather dispersibility) of certain colloidal components of the plasma membrane, the effect being referable to the adsorption of the anions. In some way this change facilitates the membrane alteration which appears to be the first stage in stimulation.

SUMMARY

When a frog's sartorious is immersed in a pure isotonic Na-salt solution for a short period, it becomes more responsive to the chemical stimulation of an isotonic NaCl solution containing 5 millimols KCl per liter.

The increase in irritability (sensitization) depends upon the nature of the anions and corresponds in degree to their relative positions in the lyotropic series; namely,

$$NaCl < NaBr < NaNO_3 < NaI < NaCNS$$

For any given Na-salt solution the increase in irritability is a direct function of its concentration. The minimal concentration of each salt necessary to produce a given increase depends, in general, upon its relative position in the lyotropic series.

The change of irritability in any pure Na-salt solution increases rapidly with the duration of immersion in that solution. The rapidity with which the maximal response is reached depends again upon the nature of the anions, and follows the lyotropic order.

The action of any given Na-salt solution is antagonized by HCl and chlorides of the alkali earth metals in the following order:

$$SrCl_2 < MgCl_2, CaCl_2 < HCl$$

The minimal concentration of any of these antagonizing substances for each Na-salt solution depends again upon the nature of the anion of the Na-salt, increasing in the lyotropic order as above.

The skeletal muscle is more susceptible to chemical stimulation at low temperature, and the sensitizing action of all the Na-salt solutions (except NaCl) is greater at low temperature; i.e., exhibits a negative temperature coefficient.

It is suggested that these Na-salt solutions tend to facilitate or increase the dispersion of certain colloidal constituents of the plasma membrane, as a result of the differential adsorption of anions, and that the increase of irritability is a consequence. The correlation with the lyotropic order of the anions is thus explained.

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THE COLD STIMULATION AND THE INFLUENCE OF NEUTRAL SODIUM-SALT SOLUTIONS ON THE COLD STIMULATION

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The production of heat rigor in skeletal muscle at high temperature is essentially an irreversible process, associated with a coagulative change of the muscle protoplasm. Reversible thermal contracture has been reported by Jensen (1909), produced by rapidly plunging a frog's sartorius through a hot Ringer's solution (or 0.6 per cent NaCl solution, or tap water, or mercury) at about 80°C. to a Ringer's solution near 0°C. This reversible thermal contracture can be obtained in curarized muscles as well as in muscles rendered non-irritable to electrical stimulation by narcosis or by previous freezing and thawing (Jensen, 1915). Jensen concluded that heat was the stimulus, but failed to appreciate the importance of the sudden large decrease in temperature.

Sommerkamp (1928) described a single observation on the production of contracture in the frog's ileo-fibularis by changing from a warm Ringer's solution at 30° to a cold one at 2° to 4°C. The present author has also had occasion to witness such contracture in a pair of apparently very irritable frog's sartorii on change from a Ringer's solution at 24° to a cold one at 0°C. These isolated observations suggest that under certain conditions cold may serve as an adequate stimulus. In fact, it has been observed that the irritability of the frog's muscle and nerve to electrical stimulation of relatively long duration is increased at low temperature (Gotch and Macdonald, 1896; Lucas and Mines, 1907). The irritability of the frog's sartorius to chemical stimulation is also increased at low temperature (Chao, 1934). In the present paper evidence will be presented to show that a sudden decrease in temperature can be made to stimulate the frog's skeletal muscle under suitable conditions and that the response of the skeletal muscle to this cold stimulation is influenced by the action of the neutral Na-salt solutions in the same way as its response to the chemical stimulation.

The procedure of experimentation is essentially the same as described in a previous paper (Chao, 1934). Experiments were performed in pairs, using in each case a pair of sartorii from the same frog. The stimulating effect accompanying a sudden decrease in temperature is made obvious in the same way that threshold, or subthreshold, chemical stimulation is

rendered effective (Lillie, 1910); namely, by increasing the irritability of the muscle by a temporary treatment with a pure isotonic Na-salt solution.

Thus, when a frog's sartorius is immersed in a pure isotonic Na-salt solution at room temperature for a short period, it becomes distinctly more sensitive to a sudden decrease in temperature. If the muscle is then suddenly transferred to a cold solution at 0°C., it gives rise to a reversible contracture while in the cold solution (fig. 1). In the following experiment a pair of sartorii were immersed in an isotonic NaBr solution at 20° for 6

Fig. 1

Fig. 2

Fig. 1. Contracture in the cold Ringer's solution at 0°C. after a period of immersion of the same muscle for 4 minutes, *a*, in a pure isotonic $NaNO_3$ solution at 20°, and *b*, in a pure isotonic NaBr solution at 20°. Note the contracture appears almost simultaneously with the change of solutions (or temperature), marked by a rise and fall of the base line in the tracings.

Fig. 2. Contracture in a Ringer's solution at 3° after a period of immersion in a pure isotonic NaCNS solution at 21° for 5 seconds.

minutes, then the NaBr solution was exchanged for various cold solutions at 0°C. The results are shown in table 1.

The above experiments show conclusively that reversible contracture can be readily produced by a sudden decrease in temperature in muscles previously sensitized with a pure Na-salt solution. Even pure isotonic $SrCl_2$ or $CaCl_2$ solution can produce contracture under these conditions.[1]

[1] The contracture produced in the cold isotonic $CaCl_2$, or $SrCl_2$, solution is lower than that in the cold Ringer's solution at the same temperature, indicating that $CaCl_2$ or $SrCl_2$ has a secondary effect in partly suppressing the full development of the cold contracture.

The contracture is not due to an exchange of anions, for under suitable conditions change from a pure isotonic NaCl solution at room temperature to a Ringer's solution at 0° has the same effect. Therefore the contracture must be a purely thermal reaction of the sensitized muscle to a sudden decrease in temperature.[2]

The contracture produced in a cold solution as a result of a sudden decrease in temperature is a function of the difference in temperature. The relation between the difference in temperature (Δt) and the height of contracture is clearly shown in table 2, which gives the results obtained on three pairs of sartorii. Each figure represents the average of two deter-

TABLE 1

Height of contracture produced in various cold solutions by a sudden decrease in temperature of 20 degrees after a period of immersion of a pair of sartorii in an isotonic NaBr solution at 20° for 6 minutes*

STIMULATING SOLUTION AT 0°C.	HEIGHT OF CONTRACTURE IN THE COLD SOLUTION IN MILLIMETERS (a) BEFORE AND (b) AFTER IMMERSION IN NaBr SOLUTION		INCREASE IN HEIGHT OF CONTRACTURE IN MILLIMETERS
	(a)	(b)	
Muscle A:			
Ringer's solution	0	100	100
M/4 dextrose solution	24	104	80
M/5 sucrose solution	50	109	59
M/10 $SrCl_2$ solution	0	55	55
Ringer's solution	0	110	110
Muscle B:			
Ringer's solution	0	111	111
M/4 dextrose solution	40	121	81
M/4 sucrose solution	61	120	59
M/10 $SrCl_2$ solution	0	69	69
Ringer's solution	0	119	119

* The height of contracture is obtained in all the experiments in this work from kymograph tracings recorded by levers of a magnification of about 7 times.

minations (one on each member of a pair of muscles). Each muscle was immersed in a pure isotonic NaBr solution for 6 minutes in every trial; from this solution it was transferred to a Ringer's solution at a different temperature.

In general, therefore, a sudden decrease in temperature under these conditions produces a contracture; a sudden increase in temperature, on the other hand, has no such effect. The least decrease in temperature necessary to produce a contracture (after a period of immersion in a pure isotonic

[2] Similar contracture can be also produced by sensitizing a curarized muscle to the cold stimulation.

NaBr solution for 6 minutes) is about 10 degrees; and the greater the decrease in temperature, the higher is the contracture.

The response to a given decrease in temperature (e.g., 10 degrees) at different temperatures, however, is not of the same magnitude. In the following experiments, muscles were immersed in pure isotonic $NaNO_3$ solutions at a given temperature (e.g., 30°, 20°, or 10°) for 2 minutes and then transferred to a Ringer's solution 10 degrees below the temperature of the $NaNO_3$ solution (i.e., 20°, 10° or 0° respectively). The average heights of contractures of 10 experiments are summarized in table 3.

TABLE 2

Relation between difference in temperature and height of contracture

	TEMPERATURE OF NaBr SOLUTION	TEMPERATURE OF RINGER'S SOLUTION	Δt	HEIGHT OF CONTRACTURE IN MILLIMETERS
(1)	20°	22°	+2	0
	20°	10°	−10	6
	20°	5°	−15	17
	20°	0°	−20	79
(2)	30°	0°	−30	98
	20°	0°	−20	75
	10°	0°	−10	60
	0°	0°	0	0
(3)	2°	31°	+29	0
	30°	25°	−5	0
	30°	20°	−10	28
	30°	10°	−20	57
	30°	0°	−30	90

TABLE 3

Response of muscle to a decrease of temperature of 10 degrees after it has been immersed in a $NaNO_3$ solution at different temperatures (average of 10 experiments)

TEMPERATURE OF $NaNO_3$	TEMPERATURE OF RINGER	AVERAGE HEIGHT OF CONTRACTURE IN MILLIMETERS
30°	20°	14
20°	10°	8
10°	0°	62

It is obvious from table 3 that the response on change from 10° to 0° is by far the more pronounced, while the response on change from 30° to 20° seems to be a little higher than that from 20° to 10°.

The production of contracture in the cold Ringer's solution depends upon the duration of immersion in the Na-salt solution. The following experiment (table 4 and fig. 3) of immersing a pair of sartorii in a pure isotonic NaBr solution at 20°C. for varying lengths of time and then transferring them to a Ringer's solution at 0°C. shows that the height of re-

sponse to the cold stimulation increases rapidly with the duration of immersion and reaches a maximum after 6 minutes in the NaBr solution.

The action of the pure Na-salt solutions in sensitizing the skeletal muscle

TABLE 4

Effect of duration of immersion in a pure isotonic NaBr solution at 20° on the height of contracture produced on transfer to a Ringer's solution at 0°

DURATION OF IMMERSION IN MINUTES	HEIGHT OF CONTRACTURE IN MILLIMETERS		
	Muscle A	Muscle B	Average
1	22	56	39
2	39	53	46
4	45	64	55
6	50	65	58
8	44	71	58

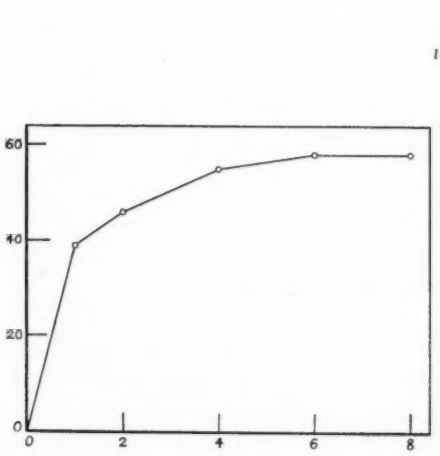

Fig. 3

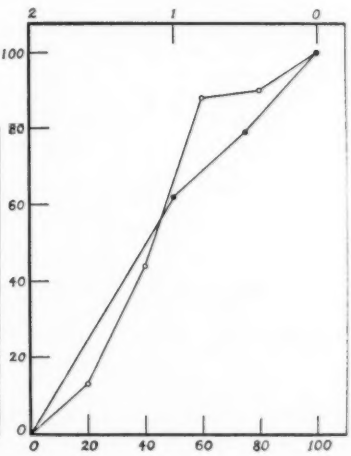

Fig. 4

Fig. 3. Relation between duration of immersion in a pure isotonic NaBr solution at 20° and the height of contracture in the cold Ringer's solution at 0°. Ordinates: height of contracture in millimeters. Abscissas: duration of immersion in minutes.

Fig. 4. *a*. Effect of concentration of NaI (circles), and *b*, effect of concentration of $CaCl_2$ (dots) on response to cold stimulation. Ordinates: percentage height of contracture. Abscissas: *a*, percentage volumes of isotonic NaI solution in Ringer's solution (lower), and *b*, millimols $CaCl_2$ per liter isotonic NaI solution (upper).

to the cold stimulation is rather rapid. Figure 2 illustrates the response of a muscle to a change of temperature of 18 degrees after a period of immersion in a pure isotonic NaCNS solution at 21° for 5 seconds only.

Considerable contracture appeared immediately on change to a Ringer's solution at 3°, and indeed the contracture approaches the shape of a single muscle twitch.

The response of the skeletal muscle to the cold stimulation, made possible by an immersion in a Na-salt solution, is a function of the concentration of the Na-salt used. Thus, in the following series of experiments a pair of sartorii were immersed in isotonic NaI solutions diluted with various amounts of a Ringer's solution at 20° for 2 minutes; the percentage heights of contractures in the Ringer's solution at 0° are given in table 5.

TABLE 5

Effect of concentration of NaI (diluted with Ringer's solution) on the response of muscle to the cold stimulation (Ringer's solution at 0°)

The response for the pure isotonic NaI solution was taken as 100 per cent.

VOLUMES PER CENT ISOTONIC NaI SOLUTION IN RINGER	PERCENTAGE HEIGHT OF CONTRACTURE		
	Muscle A	Muscle B	Average
100	*100*	*100*	*100*
80	93	87	90
60	93	83	88
40	41	47	44
20	21	5	13
0	0	0	0

TABLE 6

Effect of $CaCl_2$ on the action of NaI

The response for the pure NaI solution was taken as 100 per cent.

m.M. $CaCl_2$ PER LITER ISOTONIC NaI SOLUTION	PERCENTAGE HEIGHTS OF CONTRACTURES IN RINGER'S SOLUTION AT 0°		
	Muscle A	Muscle B	Average
0	*100*	*100*	*100*
0.5	77	80	79
1	50	73	62
2	0	0	0

The action of a pure Na-salt solution in promoting contracture on transfer of a muscle to a cold Ringer's soution is, furthermore, antagonized by the addition of $CaCl_2$. Table 6 gives the percentage heights of contractures obtained in a pair of sartorii on change to a Ringer's solution at 0° after periods of immersions of 2 minutes in NaI solutions containing various concentrations of $CaCl_2$ at 20°.

The results in table 5 and table 6 are graphically represented in figure 4. The higher the concentration of NaI, the greater is the response to the cold stimulation. On the other hand, the higher the concentration of

$CaCl_2$ in the NaI solution, the less is the response; and the sensitizing action of NaI is completely suppressed when the concentration of $CaCl_2$ reaches 2 millimols per liter.

The contracture produced in the cold Ringer's solution after a period of immersion in a pure Na-salt solution also depends upon the nature of the anions of the Na-salts. The heights of contractures produced in the cold Ringer's solution at 0° after immersions of a pair of sartorii in different pure isotonic Na-salt solutions at 20° for periods of 4 minutes are given in table 7.

Thus the order of the relative effectiveness of these Na-salt solutions in increasing the response of the skeletal muscle to the cold stimulation is as follows:

$$NaCl < NaBr < NaNO_3 < NaI < NaCNS$$

TABLE 7

Relation between the height of contracture and the nature of the anions of the Na-salt solutions

Na-salt solution at 20°C.	Height of contracture in millimeters in Ringer at 0°C.		
	Muscle A	Muscle B	Average
NaCl*	0	0	0
NaBr	83	62	73
$NaNO_3$	91	64	78
NaI	120	75	98
NaCNS	123	124	124

* Pure isotonic NaCl solution is also capable of producing contracture in the cold Ringer's solution; only in this particular experiment under these conditions it happened to be ineffective.

Discussion. Two processes are involved in this cold stimulation: 1, a preliminary treatment with a pure Na-salt solution, and 2, a sudden decrease in temperature. The effect of the former treatment, preparatory in nature, is apparently to modify the colloidal structure of the plasma membrane of the muscle fibres (e.g., to increase the dispersion of some colloidal substance) and is antagonized by the addition of a sufficient amount of $CaCl_2$. The effect of the latter treatment depends on the purely thermal phenomenon—simple decrease in temperature—and is relatively independent of the nature of the cold solution. Thus even pure isotonic $CaCl_2$ and $SrCl_2$ solutions are capable of producing a contracture under these conditions. The contracture appears immediately following the decrease in temperature and is perfectly reversible. The muscle relaxes almost completely in a short period while still in the cold solution. Therefore, whatever the stimulation process may be, the fundamental excitatory

changes in the cold stimulation are only temporary and similar in their general nature to those produced by chemical stimulation.

Furthermore, the influence of the Na-salt solutions on the cold stimulation shows, in general, a close parallelism to the influence on chemical stimulation (Chao, 1934). The lyotropic anion order of the Na-salts, the effect of duration of immersion in a Na-salt solution, the effect of concentration of a Na-salt, and the antagonistic action of $CaCl_2$—all agree, both qualitatively and quantitatively, very well with the results obtained with the chemical stimulation. In short, whatever conclusions were drawn in reference to the action of the Na-salt solutions on chemical stimulation seem to apply equally well to cold stimulation.

SUMMARY

When a frog's sartorius is immersed in a pure isotonic Na-salt solution at room temperature for a short period of time, a reversible contracture appears on sudden change to a cold Ringer's solution, or any other cold solution. Evidence has been presented to show that this contracture is produced by the sudden decrease in temperature—the stimulus of cold. The height of contracture depends upon the difference in temperature on the one hand and upon the special action of the Na-salt solution on the other. The sensitizing or facilitating influence of the Na-salt solutions on the cold stimulation is essentially identical with that on the chemical stimulation.

The author wishes to thank Dr. R. S. Lillie for valuable suggestions and much help.

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THE VISCOSITY OF THE BLOOD OF PIGEONS IN THE QUIET STATE AND FOLLOWING EXCITEMENT

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A survey of the literature on bird blood reveals insignificant data on its physical and chemical phases, although a considerable amount of work has been done on the normal cytological picture and its modification under various experimental procedures, Forkner (1929); Cash and Doan (1931).

With the view of determining the differences which may occur in the viscosity of the blood of normal pigeons in the quiet as compared to the excited state this study was undertaken. Nice and Fishman (1934) found a considerable increase in the viscosity of the blood of rats and rabbits following excitement (fear, anger, pain), when compared to the quiet state.

METHODS. The blood was obtained in the quiet state from a wing vein by puncturing it with an hypodermic needle. The viscosity was measured at 26°C. by means of a Hess viscosimeter. Excitement was then produced by restraining and teasing the bird with a weak faradic stimulus for approximately two minutes. The blood was then quickly obtained as above and its viscosity determined.

The pigeons under excitement showed all the signs of sympathetic stimulation.

These birds were deprived of food for at least 16 to 20 hours before each experiment, so as to minimize the effect of digestion upon the viscosity of the blood. Also a uniform procedure in the collection of the blood samples and in determining the viscosity was followed. We were careful not to excite the pigeon before the first sample was obtained. The viscosity of excited blood of a bird was measured approximately fifteen minutes after that in the quiet state.

RESULTS. The results of this study are shown in table 1 which represents 15 of our 27 observations. An inspection of this table reveals a marked increase in the viscosity of the blood taking place in each pigeon following excitement. The average figures for these 15 observations were 3.82 and 4.70 respectively while for twenty-seven pigeons in the quiet state it was 3.86 and following excitement 4.76 which is an increase of 23 per cent.

DISCUSSION. The above figures compare with 4.18 in the quiet state and 5.09 in the excited state for 24 normal rats; and with 3.51 and 3.95 respectively for rabbits, Nice and Fishman (1934).

TABLE 1

Measurements of the viscosity of the blood (before and after excitement)

EXPERIMENT NUMBER	VISCOSITY MEASUREMENTS	AVERAGE	PER CENT INCREASE AFTER EXCITEMENT
1	Q. 3.70, 3.55, 3.50	3.58	
	E. 3.95, 3.90, 3.90	3.92	9.5
2	Q. 4.10, 4.00, 4.00	4.03	
	E. 4.80, 4.75, 4.70	4.75	17.9
3	Q. 3.75, 3.50, 3.50	3.58	
	E. 4.10, 3.95, 3.90	3.98	11.2
4	Q. 3.50, 3.45, 3.40	3.45	
	E. 5.40, 5.15, 5.15	5.23	51.6
5	Q. 4.00, 3.90, 3.80	3.90	
	E. 5.00, 4.90, 3.85	4.91	25.9
6	Q. 3.80, 3.75, 3.70	3.75	
	E. 4.30, 4.10, 4.10	4.17	11.2
7	Q. 4.00, 3.90, 3.80	3.90	
	E. 4.70, 4.65, 4.60	4.65	19.2
8	Q. 4.70, 4.70, 4.70	4.70	
	E. 5.30, 5.30, 5.30	5.30	12.8
9	Q. 3.60, 3.50, 3.45	3.52	
	E. 4.40, 4.35, 4.35	4.37	24.1
10	Q. 3.45, 3.40, 3.35	3.40	
	E. 4.85, 4.85, 4.85	4.85	42.6
11	Q. 4.10, 4.05, 4.00	4.05	
	E. 4.90, 4.85, 4.80	4.85	19.8
12	Q. 3.65, 3.60, 3.55	3.60	
	E. 5.00, 5.00, 5.00	5.00	38.9
13	Q. 4.00, 4.00	4.00	
	E. 5.10, 5.00	5.05	26.3
14	Q. 3.70, 3.55, 3.55	3.60	
	E. 4.30, 4.20, 4.20	4.23	17.5
15	Q. 4.20, 4.20, 4.20	4.20	
	E. 5.20, 5.20, 5.20	5.20	23.8
Average.....	Q.	3.82	
	E.	4.70	23.5

Q. = quiet; E. = excitement.

As to an explanation of the mechanism involved in producing this marked increased blood viscosity following excitement, the work of Katz and Nice (1934) is significant. In a study of the changes in the chemical elements of the blood of rabbits after emotional excitement these authors found an increase in the non-protein nitrogen, urea, uric acid, preformed and total creatinine, glucose, cholesterol and hemoglobin. Also in a study on the effect of excitement on the blood volume and hematocrit changes in rabbits Nice and Katz (1934) reported a slight fall in plasma volume with an increased corpuscle volume. It is quite probable that the two factors combined, that is, a slight shift of water into the tissues, and an inpouring of corpuscles and the products of tissue breakdown into the blood stream, account for the viscosity increases of the blood during excitement.

SUMMARY

The viscosity of the blood of pigeons in the quiet state averaged 3.86 while after excitement (fear, anxiety and pain) it was 4.86.

The average increase in the viscosity of the blood of pigeons after excitement was 23 per cent. This increase is approximately of the same magnitude as previously observed by us with other animals, Nice and Fishman (1934).

The causes of the viscosity increase during excitement are considered.

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THE COMPOSITION OF EDEMA FLUID AND LYMPH IN EDEMA AND ELEPHANTIASIS RESULTING FROM LYMPHATIC OBSTRUCTION

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It is now generally accepted that, in the normal mammal, blood capillaries in practically all parts of the body leak varying amounts of protein. How concentrated the protein may be in the capillary filtrate in such a region as the skin and subcutaneous tissue is still a matter for argument, but that tissue fluid contains measurable amounts of the plasma proteins cannot be doubted. In 1931, Field and Drinker presented evidence that this extravascular protein returned to the blood vessels by the lymphatic route and under normal conditions this was the single pathway for return. It follows that in the presence of lymphatic obstruction the edema fluid or tissue fluid which accumulates must become rich in protein. In a recent paper, Drinker, Field and Homans (1934) described the production of edema and elephantiasis in dogs by a gradual obliteration of the lymphatics in the hind legs. In such animals it is possible to observe the changing composition of the edema fluid as lymphatic obstruction becomes more and more complete, and to gain information as to the character of the medium in which fibrotic change unfailingly appears.

It is a curious fact that the chemical composition of the fluid—particularly the protein content—which accumulates when lymphatics are obstructed has had so little attention. One may cite a great deal of cytological and particularly of bacteriological data upon cases of lymphatic blockage, but chemical work is practically lacking and that which can be quoted is not certainly reliable as to method.

Desjardins (1854) collected fluid from the skin in a case of tropical elephantiasis. He found 4.3 per cent of protein. Dahnhärdt (1866) took similar fluid from a case of elephantiasis following erysipelas. He found 3.4 per cent of protein. Vecchi (1912) studied the composition of hydrocele fluid from 32 cases. Twenty-five of these were idiopathic in origin and probably were instances of lymphatic obstruction. From the cytological reports, all presented chronic conditions and were non-inflammatory when the fluid was aspirated. The protein content varied between 3.0

and 9.1 per cent, with an average of 6.33 per cent. Protein was determined refractometrically. Our own experience has furnished four cases:

1. 26 years. White. Female. Diagnosis: Elephantiasis, lymphangiectatic, right leg.

Five years prior to examination, the patient, born in Nova Scotia and now living in Massachusetts, noticed a non-tender swelling in the region of the right internal malleolus. There was no evidence of infection at that time or later, and the leg has never been painful in any part. The swelling has increased slowly ever since the onset and the entire leg is now markedly enlarged. It pits on pressure. A year ago, following removal of a splinter, clear fluid drained from the wound for 48 hours.

Fig. 1

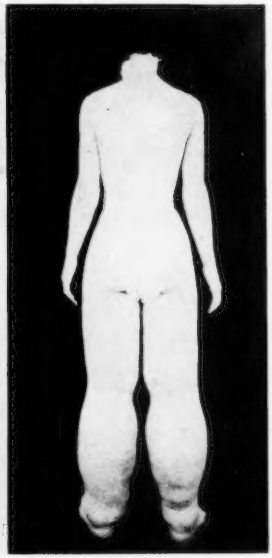

Fig. 2

Fig. 1. Patient 2. Edema and elephantiasis of the left leg. Three years' duration.
Fig. 2. Patient 4. Bilateral edema and elephantiasis. Six years' duration in right leg, four and one-half in left.

On two exploratory operations, first in the lower leg and later in the thigh and groin, preceded by trypan blue injections, the superficial tissues were everywhere sclerosed and no draining lymphatics could be found. The case was apparently one of idiopathic lymphatic obstruction uncomplicated by infection. Edema fluid removed by puncture from the inner side of the lower leg after a period of dependence showed 2.7 per cent of protein.

2. 61 years. White. Male. Diagnosis: Lymphedema, lues.

Three years before examination, the patient, a native and resident of Massachusetts, experienced a painless swelling of the lower left leg. Neither at that time nor later could any cause for this be found. There has been no history of pain nor

tenderness and nothing to indicate local infection. The Wassermann reaction was strongly positive. On examination the leg showed a brawny edema. The small ulcers in the skin below the knee were felt to be luetic. Nothing was found on examination of the pelvis and the case was thought to be one of idiopathic edema with elephantiasis.

Subcutaneous fluid flowed easily from punctures over the internal malleolus and front of the ankle. The first specimen contained 3.5 per cent of protein and the second 4.0 per cent.

Figure 1 is a photograph of the patient at the time of fluid withdrawal.

3. 32 years. White. Female. Diagnosis: Idiopathic edema, left leg.

Twenty-two years prior to examination swelling of the left leg was noticed. This has been progressive. At no time has the patient experienced acute infection or pain in the part. At present the entire left leg is considerably larger than the right. The lower leg is tense and brawny and shows a drying vesicular dermatitis. The circulation is apparently normal. The case was considered an instance of idiopathic edema. As there had been symptoms referable to the appendix the abdomen was opened and appendectomy performed. At the same time the left iliac vessels were exposed. Several dilated lymphatic trunks were seen and from one of these clear fluid was withdrawn with a syringe. Venous blood was collected at the same time.

TABLE 1

The composition of plasma, lymph and fistula fluid from case 3

	PROTEIN	NON-PROTEIN NITROGEN	SUGAR	URIC ACID	CHLORIDES
	gm. per 100 cc.	*mgm. per 100 cc.*			
Plasma	7.8	21.7	147	4.3	618
Lymph	5.5	25.6	226*	4.4	642
Fistula fluid	4.0				

* It is possible that this high sugar value is due to large amounts of non-fermentable reducing substances in the lymph.

Nine days after operation a minute fistula opened in the left groin just below Poupart's ligament. A slightly turbid fluid was collected. Analyses are found in table 1.

4. 19 years. White. Female. Diagnosis: Elephantiasis bilateral.

Six years prior to examination the patient noticed that her right ankle and instep were swollen. She could assign no cause for this and there was no pain, redness nor infection. During 1½ years the right leg became involved to the knee, the swelling increasing progressively. Four years ago the right leg suddenly became painful, particularly in the popliteal space and in the calf. This lasted for 10 days, and at this time the patient noticed her left instep was swollen. As in the case of the right leg, there were no symptoms with this first swelling. It, however, was steadily progressive. Three years prior to admission there was a second attack of pain in the right leg accompanied by malaise and fever—apparently a typical attack of lymphangitis. Shortly following this attack the patient became pregnant and both legs swelled enormously. After delivery—by Caesarean section—the legs lost a great deal of swelling, but when the patient began to get about the legs again swelled rapidly. At the present time physical examination shows nothing except an edematous left labium majus with brawny edema of both legs and elephantiasis. These changes, as indicated by figure 2, extend to the groin.

May 12, 1934, a.m. Edema fluid, collected by needle puncture from the subcutaneous tissue on the inside of the left leg, was quite clear. It contained 3.34 per cent protein and 500 white cells per cubic millimeter. On microscopic examination these were lymphocytes.

May 12, 1934, p.m. Twelve cubic centimeters of clear fluid were collected from puncture in back of left calf, 8 cm. above ankle fold. The composition of the fluid compared with that of venous blood is shown in table 2. The noteworthy feature of the fluid is the high albumin/globulin ratio. Reference to table 3 will indicate that this ratio in the dogs with edema and elephantiasis tends to be higher than in the plasma but the difference is never so great as has been encountered in this patient.

May 14, 1934. The puncture hole in the left calf has drained steadily since the afternoon of May 12. The region around the draining point is loose and baggy. It does not pit. The fluid which runs out steadily is easily collected and quite clear. It contains 3.1 per cent of protein.

TABLE 2

The composition of plasma and edema fluid from case 4

DATE	SUGAR (MGM./100 CC.)		NON-PROTEIN NITROGEN (MGM./100 CC.)		CHLORIDES (MGM./100 CC.)		PROTEIN (GM./100 CC.)		ALBUMIN (GM./100 CC.)	
	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid
May 12, 1934....	97	106	24	30	675	709	6.98	3.23	4.02	2.27
May 19, 1934....				30			7.25	2.94	4.02	1.97
May 19, 1934....								2.91		2.06

DATE	GLOBULIN (GM./100 CC.)		ALBUMIN/ GLOBULIN		COLLOID OSMOTIC PRESSURE (MM. H_2O)		COLLOID OSMOTIC PRESSURE PER GRAM PROTEIN (MM. H_2O)		
	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	
May 12, 1934....	2.96	0.96	1.36	2.36	306	162	43.9	50.1	Left leg
May 19, 1934....	3.23	0.97	1.25	2.03					Left leg
May 19, 1934....		0.85		2.42					Right leg

May 19, 1934. Edema fluid was collected from the lower part of the calf in the left and right legs. The left leg had been suspended for some time prior to this and the larger part of the fluid had drifted away from the region of puncture in the calf. The leakage which occurred after the puncture of the left calf on May 12, together with the drainage gained by elevation of the leg are probably responsible for the slight decrease in protein concentration in the edema fluid which took place between May 12 and May 19.

May 22, 1934. The pelvis was explored. Lymphatics over the right and left external iliac vessels were clearly visible and thick-walled. A drop of lymph obtained from one of those on the right side by means of a syringe, contained 2.7 per cent of protein. It was the impression of the operator that there was little or no lymph drainage through the deep lymphatic trunks.

These four cases, together with such references as we have obtained, point strongly to the function of the lymphatic system in removing extra-

vascular protein. This indication is clearly confirmed by the experimental observations which follow.

Experimental. The method for producing lymphatic obstruction was that described in a previous paper (Drinker, Field and Homans, 1934) and animals numbers *1* and *2* of that paper are *1* and *2* in this one. The development of swelling and edema in these two dogs is shown in figures 2 and 3 of the above-mentioned paper. As soon as a definite doughy edema is established, one can collect fluid by allowing the edematous leg to be dependent and quiescent for a little time and then puncturing with a small syringe needle.

The first fluid obtained is usually bloody, but in a short time becomes clear. When edema is well established the fluid may be extraordinarily plentiful. In dog 1, for example, on March 23, 1934, under nembutal anesthesia, tissue was taken from the inner side of the right ankle. During a brief operation, 300 cc. of fluid were collected from the wound. Not more than 10 cc. of this was blood. It is thus possible in dogs, where this type of edema is established more rapidly than is ordinarily true in human cases, to collect amounts of fluid large enough for all chemical requirements. Late in the process one cannot often collect lymph since draining lymphatics have been obliterated, but early in the condition a vessel large enough to cannulate may be found. This has been our good fortune several times and has enabled us to compare the composition of lymph and edema fluid.

The data to be presented concern first, and to us most important, the protein concentration of edema fluid and of lymph, second the general chemical composition of edema fluid and venous blood, and third the cytology of the edema fluid. All of these data should be correlated with an histological description of the development of elephantiasis for which we possess the material. But in the space of a single paper this is not possible and must wait a further communication.

The chemical composition of edema fluid and of lymph. To determine total protein, the Zeiss Dipping Refractometer was used, and in numerous instances was checked by Kjeldahl determinations. A curve for protein concentrations determined for our instrument is given in figure 3. This curve has been obtained by the use of ordinates depending on Kjeldahl quantitations of total protein and values upon the abscissa which are scale readings on the refractometer for the same specimens. The tables supplied with the instrument give low values when used for lymph and edema fluids. Employing the above curve, our results for subcutaneous lymph and edema fluid have an average error of 4.55 per cent for lymph and 6.05 per cent for edema fluid when compared with chemical quantitations.

The protein and cell content of the edema fluid taken from the left hind leg of dog 1 between February 25, 1933 and April 7, 1934 are shown in figure 4. In this animal the first injection of silica was given on December

12, 1932. Swelling of the leg occurred at once and though never allowed to subside completely, lymph drainage did not cease until March 25, 1933, after which date lymphatic trunks could no longer be found. On February 25, 1933, edema fluid showed 2 per cent of protein. There was a final injection of silica and quinine on March 25, indicated by the first arrow on the abscissa, figure 4. The protein in the edema fluid then rose slowly to almost 3 per cent, but in April fell once more. On May 5 this dog became ill and probably had what has since become a common experience. He was lame in his edematous leg, refused food, and was obviously sick. At the time it had not occurred to us that lymphatic obstruction alone would

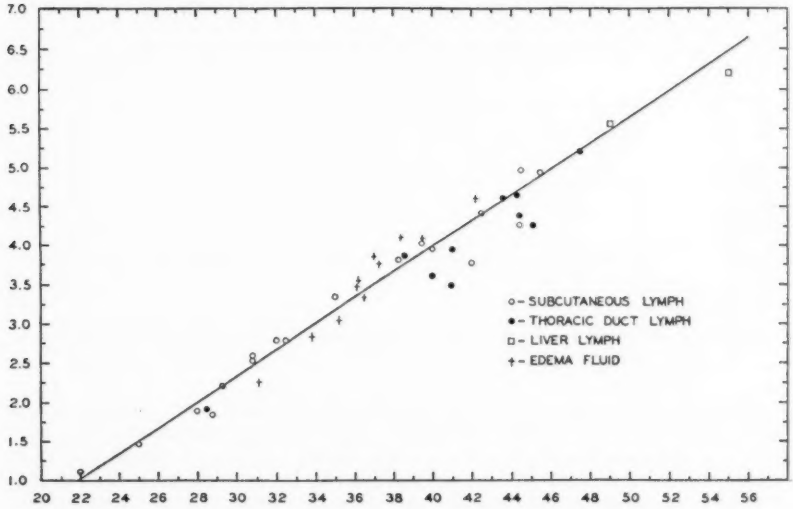

Fig. 3. Curve giving protein concentrations obtained by Zeiss Dipping Refractometer. All points determined by Kjeldahl minus non-protein nitrogen determinations on lymph and the fluid from cases of edema following lymphatic obstruction. *Ordinates*, per cent of protein; *abscissae*, scale readings on refractometer.

produce a situation favoring infection with a hemolytic streptococcus. The records of human cases of edema and elephantiasis might have prepared us for this development, but on the first occasion we failed to appreciate what was happening. After May 5, the edema fluid protein rose, and on May 15, when the second attack occurred, it reached 3.7 per cent. Subsequent attacks caused abrupt increases in protein content followed by subsidence, but never to 2 per cent. After September 18, 1933, the edema fluid protein became fixed in the neighborhood of 4 per cent. The attack on April 4, 1934 was induced by injection of a dilute culture of hemolytic streptococci isolated in a previous spontaneous attack. When this or-

ganism is injected into the edematous leg of dog 1, it invariably causes a severe reaction throughout the entire leg, with high fever. Each attack is accompanied by heat and redness. The animal lies quietly and protein always accumulates in the part. The immediate increase is largely lost within a few days as the animal becomes active and changes his position frequently. But the general effect of the acute inflammatory experience is to leave some protein behind and to intensify the condition imposed by lymphatic blockage alone.

The movements of proteinized extravascular fluid in an animal with blocked lymphatics depend largely upon gravity. On histological examination of skin and subcutaneous tissue taken from dog 1 on March 23,

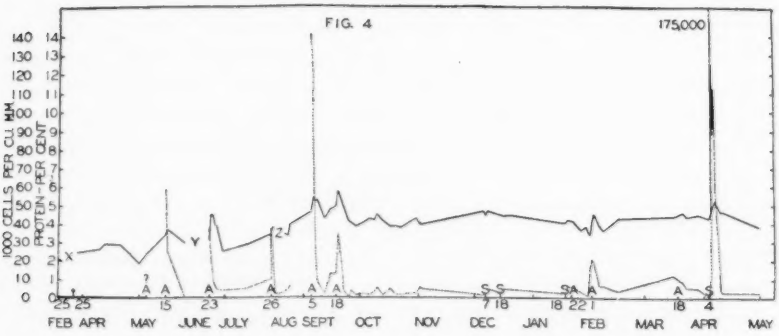
FIG. 4
175,000
1000 CELLS PER CU. MM.
PROTEIN PER CENT
140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
X
Y
Z
A A A

can watch the drift through the cutaneous lymphatics, some of which have no covering save epithelium. Eventually the fluid reaches regions where valved lymphatics exist and it then cannot flow back into the leg when the part becomes dependent. It may be wondered that the concentration of protein in the edema fluid of such an animal as dog 1 does not reach that of the blood. In our opinion this does not take place because as the dog moves about, lies down, and changes the position of his edematous leg, a certain amount of the fluid spills over into regions on the back and abdomen where competent lymphatics exist, and such fluid is carried away. In all probability a hydrocele would experience less of this type of loss, and this may account for the very high protein reported by Vecchi (1912).

Figure 5, constructed just as was figure 4, shows the protein and cell content of edema fluid collected from dog 2, the same animal as dog 2 in

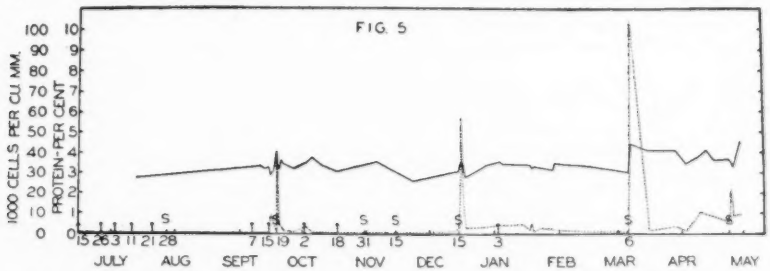

Fig. 5. *Solid line*, per cent of protein in the edema fluid of dog 2; *broken line*, cell counts in thousands per cubic millimeter. *Ordinates*, per cent protein and cells per cubic millimeter in thousands; *abscissae*, days. Arrows on abscissa indicate intralymphatic injections of silicon dioxide and 2.5 per cent quinine hydrochloride. The letter *S* indicates an inflammatory attack induced by subcutaneous injection of an hemolytic streptococcus isolated from dog 1 during a spontaneous attack.

the previous paper by Drinker, Field and Homans (1934) on the production of lymphatic obstruction and elephantiasis. The development of swelling in this animal is found in figure 3 of that paper. The first intralymphatic injection of silica and quinine was given this dog on June 15, 1933. There were then three others—June 26, July 3, and July 11—before the leg became permanently swollen. Clear edema fluid was first obtained on July 13, and contained 2.8 per cent of protein. Following blocking injections on September 7 and 15, 1933, the leg swelled rapidly and has remained large ever since. On histological examination the skin and subcutaneous tissue showed dilated lymphatics with thickened walls. In this animal fibrosis has become denser than in the case of dog 1. The protein in the edema fluid has remained around 3.0 per cent until March 6, 1934, when a subcutaneous injection of hemolytic streptococci produced a sharp inflammatory attack. Another of these occurred on April

25, 1934. These two seizures have apparently resulted in a sustained increase in the edema fluid protein. It must be understood that acute attacks of this sort last not more than two days. The edema fluid practically invariably becomes sterile in the first twelve hours. There is no evidence either local or general that a continuous inflammatory process is going on, and between attacks the edema fluid does not contain polymorphonuclear leucocytes. The cells found are lymphocytes and large mononuclear phagocytes.

Figure 6, similar to figures 4 and 5, deals with dog 3. Prior to April 28, 1933, when the first quantitation of protein in the edema fluid was made, there were seven intra-lymphatic injections of silica and quinine. On April 28, 1933, the leg was markedly swollen and showed a characteristic doughy edema. The protein concentration in the fluid remained around 3 per cent until August, 1933, when a fall occurred. This fall was syn-

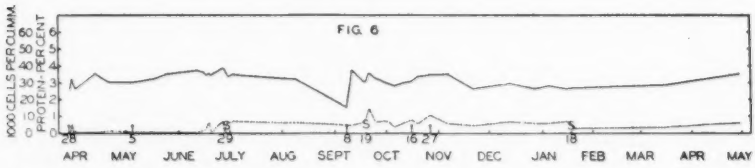

Fig. 6. *Solid line*, per cent of protein in the edema fluid of dog 3; *broken line*, cell counts in thousands per cubic millimeter. *Ordinates*, per cent protein and cells per cubic millimeter in thousands; *abscissae*, days. Arrows on abscissa indicate intra-lymphatic injections of silicon dioxide and 2.5 per cent quinine hydrochloride. The letter *S* indicates a subcutaneous injection of an hemolytic streptococcus isolated from dog 1 during a spontaneous attack of acute inflammation in a lymphedematous leg.

chronous with a series of fine fistulae which have appeared at intervals in this animal. They were of pinhole size and led into skin lymphatics which, owing to gradual dilatation, had finally lost all covering except epithelium. These fistulae drained steadily and sometimes in large amounts, so that though the dog was losing protein he was also experiencing lymph drainage and the tissue fluid environment of the part was at least steadily renewed. The intra-lymphatic injection of silica and quinine on September 8, 1934, followed by a subcutaneous injection of hemolytic streptococci on September 19, 1934, produced a more stable situation, and though fistulae occurred during the following period, the protein concentration of the edema fluid never fell much below 3 per cent. This animal has had no spontaneous inflammatory attacks. He has received two injections of the streptococcus used in preceding experiments. The first of these produced a very slight reaction and the second nothing at all. These findings are of possible interest in relation to the existence of fistulae and the greater degree of

The composition of plasma and edema fluid from dogs 1, 2, and 3

	DATE	SUGAR (MGM./100 CC.)		NON-PROTEIN NITROGEN (MGM./100 CC.)		CHLORIDES (MGM./100 CC.)		CALCIUM (MGM./100 CC.)		INORGANIC PHOSPHORUS (MGM./100 CC.)		PROTEIN (GM./100 CC.)		ALBUMIN (GM./100 CC.)		GLOBULIN (GM./100 CC.)		ALBUMIN/GLOBULIN		COLLOID OSMOTIC PRESSURE (MM. H_2O)		COLLOID OSMOTIC PRESSURE PER GRAM PROTEIN (MM. H_2O)		
		Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	Plasma	Edema fluid	
			126–128																					Left leg
Dog 1	Feb. 25, 1933											6.08	2.82	2.46	1.25	3.62	1.57	0.68	0.79					Left leg
	July 13, 1933			40.0	38.0							6.08	2.25	2.46	0.98	3.62	1.27	0.68	0.77					Right leg
	July 13, 1933			40.0	50.0							6.42	3.75	2.85	1.64	3.57	2.11	0.79	0.77					Left leg
	July 26, 1933 (1st day of attack)	97	95	19.0	18.4							6.88	4.08	2.90	1.75	3.98	2.33	0.73	0.75					Left leg
	July 27, 1933 (2nd day of attack)	91	105	23.5	26.8																			Left leg
	Aug. 24, 1933		106									7.3	4.4							231	157	32	36	Left leg
	Dec. 20, 1933	99	106	34.0	37.0							8.13	4.11	2.82	1.76	5.31	2.35	0.53	0.75	310	114	38	28	Left leg
	Feb. 7, 1934				21.0							7.12	4.58	2.68	1.57	4.44	3.01	0.62	0.52	291	122	41	27	Left leg
	Feb. 15, 1934			29.5																				
	May 4, 1934	93	110	29.3	28.4	690	710	10.6	8.8	3.8	3.8	7.38	4.15	2.78	1.63	4.60	2.52	0.61	0.65	350	171	47	41	Left leg
													3.04		1.93		1.11		1.74					Right leg
Dog 2	July 29, 1933 (Day after induced attack)				30							7.30	3.33	3.44	1.86	3.86	1.47	0.89	1.27	297	96	41	29	Left leg
	Feb. 15, 1934																							
	Apr. 30, 1934	104	113	30.0	31.0	697	716	11.1	9.5	4.2	4.0	6.57	4.08	4.15	2.21	2.42	1.87	1.71	1.18	288	119	44	29	Left leg
																								Left leg
Dog 3	June 30, 1933 (Day of streptococci inj.)	84	98	40	52.3							6.99	3.85	3.24	2.15	2.74	1.70	1.18	1.27					Left leg
	July 1, 1933 (Day of streptococci inj.)	80	101	40.0	50.4							6.73	3.53	3.11	1.97	3.62	1.56	0.86	1.27					Left leg
													3.48		2.07		1.41		1.46					Left leg
	Aug. 9, 1933				75.0																			Right leg
	Apr. 6, 1933	123	113	46.9	39.7	673	700	12.2	8.9	4.0	3.6	7.11	2.71	4.24	1.77	2.87	0.94	1.48	1.88	340	112	48	41	

renewal of proteinized extravascular fluid than has been the case in other animals.

In table 3 is found a more detailed description of the edema fluid taken from dogs 1, 2 and 3 on different occasions and compared with venous blood secured at the same time. The quantitative methods used were as follows:

Sugar. Folin, 1929.
Non-protein nitrogen. Folin and Wu, 1919.
Chlorides. McLean and Van Slyke, 1915.
Calcium. Fiske and Logan, 1931.
Inorganic phosphates. Fiske and Subbarrow, 1925.
Protein. Kjeldahl.

TABLE 4

The protein content of lymph and edema fluid from dogs 1, 2, 3, and 4. Blood and lymph collected simultaneously.

ANIMAL	DATE	LEG	PROTEIN IN GRAMS PER 100 CC.	
			Lymph	Edema fluid
Dog 1	Feb. 25, 1933	Left	2.67	2.00
	Mar. 8, 1933	Right	2.45	2.20
Dog 2	Sept. 15, 1933	Left	3.37	3.37
	April 3, 1934	Left	2.48	3.45
Dog 3	Mar. 28, 1933	Left	2.28	2.75
	May 5, 1933	Left	2.97	3.17
	Oct. 16, 1933	Left	3.17	3.17
	April 6, 1934	Right	2.50	2.67
Dog 4	April 9, 1934	Left	2.55	1.86

Albumin and globulin. Separation by precipitation of globulin with 22.2 per cent sodium sulphate. Nitrogen determined by micro-Kjeldahl analysis, a modified Pregl apparatus being used for distillation.

Colloid osmotic pressure. Method of Krogh and Nakazawa as described by Turner, 1932.

Table 4 is a comparison of the protein content of lymph and edema fluid collected from the foot and lower leg of dogs 1, 2, 3 and 4. In these samples, an effort was made to obtain the edema fluid from the region drained by the lymphatic which was cannulated to yield lymph. We had expected to find a greater degree of identity between the two fluids, but after the lymphatics become dilated and thickened and the vascularization of the part is altered by advancing fibrosis, the edema fluid protein may vary in different parts of the same leg. For example, on April 17, 1934, edema

fluid was collected from four places in the left leg of dog 2. The results were as follows:

Dorsum of foot, 3.17 per cent protein.
Outside of leg at ankle, 3.45 per cent protein.
Five centimeters higher, 3.62 per cent protein.
Inside of leg, 3 cm. above ankle, 3.50 per cent protein.

The cytology of the edema fluid. When the lymph drainage of a part is obliterated we have seen that a highly proteinized fluid accumulates. This fluid clots. It contains varying numbers of cells and fragments of eosin staining material, the origin of which cannot be ascertained. Except at the time of an inflammatory attack the cells are in the main lymphocytes with the addition of a few large mononuclear cells which stain vitally and are unquestionably wandering macrophages. In figures 4 and 5, cell counts in the edema fluid are observed to rise abruptly with the attacks of inflammation. These increases are due to a sudden outpouring of polymorphonuclear leucocytes. This outflow coincides with the onset of fever and is astonishingly rapid.

A typical cross-section of the findings as to cells is given by dog 2 beginning December 15, 1933. On that day an acute attack was induced by subcutaneous injection of a dilute culture of hemolytic streptococci. The cell content of the edema fluid prior to injection and after the acute attack had subsided is given in table 5. Between attacks the edema fluid practically always contains cells. These for the most part stain badly and cannot be identified with certainty. With an attack there is a tremendous outflow of polymorphonuclear leucocytes which soon begin to stain poorly and eventually become quite unrecognizable. Large mononuclear cells become numerous as the attack subsides. One can find them at practically all times but they are usually badly stained, the nucleus is ill-defined, and attempts at vital staining are of no avail. In a later paper on the bacteriology of the edema fluid other details as to cytology will be presented. At this time it is our aim to record the composition of the edema fluid in which increase in fibrous tissue occurs. This fluid contains a high concentration of blood proteins with unidentifiable fragments of protein and a varying number of white blood cells more or less disintegrated. With these a few red cells are found, but if care is taken in collecting the fluid they are not numerous.

Discussion. The findings we have listed need little comment. There is but one point which we should like to emphasize. It has been necessary to mention attacks of chills and fever, and the impression may have been gained that the environment of the edematous part in these animals has been steadily influenced by inflammation. This is not the case. The attacks of inflammation, either spontaneous or induced, are transient and the irritation induced by the sterile injections of quinine and silica is even

TABLE 5

The cytology of the edema fluid from dog 2, before, during and after the acute inflammatory attack of December, 1933

DATE	HOUR	WHITE CELLS PER CU. MM.	POLYMORPHONUCLEAR NEUTROPHILS	LARGE MONONUCLEARS	LYMPHOCYTES	UNIDENTIFIABLE CELLS	REMARKS
1933	*a.m.*						
Dec. 15	9:45	2,400	0	2	7	91	Size of cells and characteristics of nucleus render it probable unknown types of cells are lymphocytes
	11:00	10 cc. of a culture of hemolytic streptococci containing 250 colonies per cubic centimeter injected subcutaneously in left leg. Three points of injection					
	p.m.						
	2:30	1,600	0	0	1	99	Unidentifiable cells probably lymphocytes
	6:30	1,900	1	0	0	99	Unidentifiable cells probably lymphocytes
	11:25	2,600	2	2	0	96	Unidentifiable cells probably lymphocytes
	a.m.						
Dec. 16	3:50	2,500	0	2	1	97	Unidentifiable cells probably lymphocytes. Animal entirely normal. Rectal temperature 101.4
	8:15						Lame in left leg and unwilling to move
	8:20						Hard chill. Rectal temperature 103.8
	8:40	35,800	90	0	0	10	Cells not identifiable are in the main polymorphonuclear in size
	9:30						Rectal temperature 104.8
	11:25	57,400	80	1	0	19	Cells not identifiable are probably polymorphonuclears
	p.m.						
	3:30						Rectal temperature 102.5
	3:45	43,400	85	5	1	9	Large mononuclear cells quite numerous and often phagocytic for leucocytes
	8:10	29,500	55	0	0	45	Unidentifiable cells probably polymorphonuclears. Rectal temperature 102.4
	a.m.						
Dec. 17	8:55						Rectal temperature 101.4. No limp
	11:30	14,300	33	9	0	58	
Dec. 18	8:30						Rectal temperature 100.9
	9:20	2,900	2	8	8	82	Unidentifiable cells lymphocytes in size
Dec. 28	10:00	3,700	0	0	0	100	Unidentifiable cells stain badly and are very variable in size

more so. It is true that inflammation increases protein in the edema fluid and brings in large numbers of cells. But with lymphatic blockage alone the edema fluid protein may rise above 3 per cent and the cells in the fluid increase. Figure 7 shows the characteristics of the cells in the edema fluid before, during, and after an attack in dog 2, table 5.

Our experience points clearly to the fact that the principal function of the lymphatic system is to drain off extravascular protein. Lacking this drainage, cellular environment becomes abnormal and fibrosis begins.

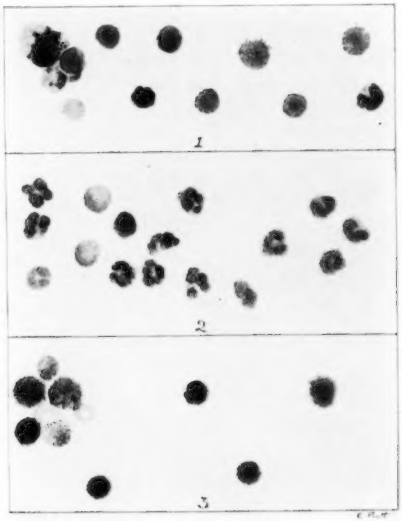

Fig. 7. Camera lucida drawings of three smears of edema fluid from dog 2. The cytology of the smears is given in table 3. No. 1, December 16, 1933, 3:50 a.m. No. 2, December 16, 1933, 8:40 a.m. No. 3, December 18, 1933, 9:20 a.m. All ×1300.

SUMMARY

By means of curves and tables, an account has been given of the chemical and cytological characteristics of edema fluid taken from three dogs with what is, as far as one can tell, complete lymphatic obstruction in the hind leg. Under these circumstances the proteins in the edema fluid may reach a concentration of 4.0 to 5.0 per cent. The edema fluid invariably contains cells. Except at times of spontaneous or induced attacks of infection when polymorphonuclear leucocytes predominate, the cells are mainly lymphocytes, and these show a poorly defined nucleus and cytoplasm and are in the main degenerating forms. The albumin/globulin ratio tends to be slightly higher in edema fluid than in blood but the difference is not great.

When the protein content of lymph and edema fluid are examined together the results show a fair degree of similarity.

The systematic findings from dogs with edema due to lymphatic block follow the expectations one gains from the meagre data in the literature upon human cases and such instances as we have been permitted to examine. For the four latter we are indebted to Dr. John Homans of the Peter Bent Brigham Hospital and to Dr. James C. White of the Massachusetts General Hospital.

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THE EFFECT OF HISTAMINE ON JEJUNAL SECRETION

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While considerable data have been accumulated on the effect of histamine on the secretory activity of the stomach of the dog, the cat and man, relatively little is known of its effect on the secretory activity of the intestine. Koskowski (1) showed that the subcutaneous administration of histamine to dogs stimulated the glands in the small intestine to secretory activity after a very short latent period. Nechoroschew (2) confirmed this observation and found that even when the histamine was administered through a duodenal fistula the secretory activity of the small bowel was increased.

We have investigated the secretion of intestinal juice into jejunal loops following the subcutaneous injection of histamine diphosphate in fasting, unanesthetized dogs.

METHOD. The dogs used in these experiments had previously been prepared by the Johnston (3) modification of the Thiry (4) loop. Each loop was approximately 22.5 cm. in length. The dogs weighed from 12 to 27 kgm.

At the time of the experiment a Johnston (3) catheter was inserted in the jejunal loop. The loop was carefully irrigated and evacuated, following which the collection of intestinal juice under normal conditions in the loop was begun.

CONTROL EXPERIMENTS. In order to determine the normal rate of secretion under experimental conditions, eight experiments were continued for three hours, the secretion being withdrawn and measured at thirty minute intervals. The results of these control experiments are plotted as curves in figure 1.

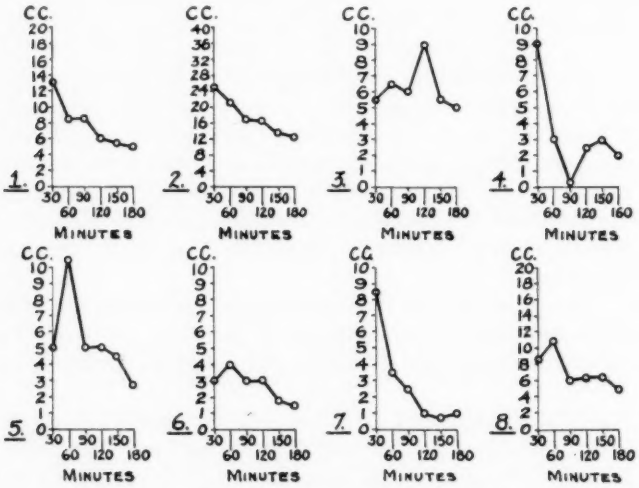
The curves in figure 1 in general show a tendency to a decrease in the rate of secretion during the three hour period. Although the general trend of the rate of secretion was similar in all experiments, the actual volumes obtained varied considerably in different dogs and even in the same dog on different days.

HISTAMINE EXPERIMENTS. In these experiments the jejunal secretion was collected and measured under control conditions for one or two thirty-

[1] Aided by a grant from the Faculty Research Committee.

minute periods, following which 3.0 cc. of a 1:1,000 solution of histamine diphosphate were injected into the subcutaneous tissues of the anterior abdominal wall. The withdrawal of fluid was repeated at thirty minute intervals for as long as two hours following the histamine injection. The results of these experiments are recorded in table 1.

The volumes of juice secreted during the control periods were subject to wide variations in the different dogs and even in the same dog on different days, just as in the control experiments. However, following the injection of histamine there was invariably a temporary increase in the volume of secretion, the extent of which varied as much as did the control volumes.



VOLUMES SECRETED DURING CONTROL EXPERIMENTS

Fig. 1

Some of the intestinal juice secreted by these dogs was examined by Dr. F. A. Cajori of the Department of Physiological Chemistry (5) for its enzyme content. The results of his experiments indicated that histamine had no apparent effect upon the production of the enzymes amylase, ereptase or sucrase.

DISCUSSION. Only four references have been found in the literature on this subject. Koskowski (1), Nechoroschew (2) and Starling (6) have studied the effect of histamine on the secretory activity of the intestine of dogs, while Savitsch (7) worked with human beings. All report an increase in intestinal secretion following histamine, although very few data are recorded.

Better known is the action of histamine on the other glands of the digestive tract. The literature is especially rich in data on the effect of histamine on the gastric glands.

It is of interest to compare the effect of histamine on the gastric and intestinal glands.

TABLE 1

Volumes of intestinal juice secreted into jejunal loops before and after injections of histamine

DOG NUMBER	CONTROL PERIOD		FOLLOWING HISTAMINE			
	First 30 minutes	Second 30 minutes	First 30 minutes	Second 30 minutes	Third 30 minutes	4th 30 minutes
	cc.	cc	cc.	cc.	cc.	cc.
444	26.0	35.0	33.0	27.0		
	0.0	0.0	12.0	6.0		
	8.0		22.0	9.0		
	13.0	10.5	25.0	13.0		
	15.0		26.0	35.0		
	10.0		16.0			
	20.0		24.0	20.0	15.0	11.0
36	28.0	29.0	32.0	17.0		
	11.5		26.5			
	6.0		31.0	10.0		
	8.5	8.5	13.5	6.0		
	6.0		14.0			
	18.0		34.0			
	15.0		17.0			
	8.5		14.0			
	2.0		5.0			
	8.5		18.0			
266	20.5		44.0			
	10.0		16.0	4.0		
	9.0		16.0	23.0	7.0	5.0
941	12.0		19.0	7.0		
	16.0		33.0	20.0	16.0	8.0
	10.0		27.0			
	12.0		30.0			
585	12.0		17.0			
	7.0		30.0			

Fluid volume. Bloomfield and Polland (8) in reporting the action of histamine in increasing the total secretion of the stomach in a series of experiments made upon normal humans, have presented their results in graphic form.

For comparison of the results found by Bloomfield and Polland and those found in the present experiments, the figures which Bloomfield and Polland used in preparing their graph as well as our own data have been converted to percentages (fig. 2).

The striking similarity between the two curves lends considerable strength to the suggestion that, as far as the total volume of secretion is concerned, the effect of histamine upon the jejunum is the same as that upon the stomach.

Enzyme production. The literature on the effect of histamine on the production of pepsin by the stomach is not in agreement. A large group of observers (9 to 22), who have for the most part used human subjects, report that histamine increases the output of pepsin. On the contrary, Babkin and Vineberg (22) and Gilman and Cowgill (23) using dogs report no increase in total output of pepsin. The observations of Cajori (5) on our animals, that histamine is apparently without effect on the total enzyme

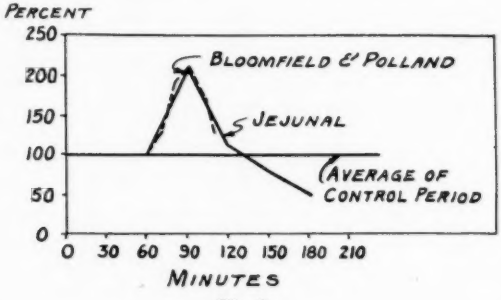


Fig. 2

production of the jejunum, would compare favorably with the findings of the latter authors on the effect of histamine on pepsin production.

SUMMARY AND CONCLUSIONS

The rate of secretion of intestinal juice in unanesthetized dogs with jejunal loops before and after the administration of histamine has been studied.

The normal rate of secretion of intestinal juice by jejunal loops varied remarkably in different dogs and also in the same dog on different days. There occurs as a rule a gradual, progressive decrease in the rate of secretion during the period of observation under control conditions.

The subcutaneous injection of a standardized dose of histamine diphosphate produces invariably a temporary increase in the rate of secretion. The extent of this increase varies in different dogs and in the same dog on different days, but the average of a series of experiments shows that the

increase reaches its height at thirty minutes after the injection, after which time the rate decreases.

The data of Cajori (5) based upon the secretion obtained from the dogs used in these experiments indicate that histamine diphosphate has no apparent effect upon the production of enzymes by the jejunum.

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